Posters

New antimicrobials – before and after entering clinical development

P532 Antimicrobial activity of telavancin against *Enterococcus faecalis*, *E. faecium* and *E. avium*: results from a European surveillance programme (2007)

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Objectives: To evaluate the potency of telavancin (TLV) against enterococcal isolates (*Enterococcus faecalis* [EF], *E. faecium* [EFM] and *E. avium* [EAV]) collected as part of a European surveillance protocol for 2007. TLV is an investigational, intravenous, semi-synthetic, rapidly bactericidal lipoglycopeptide that is broadly active against both aerobic and anaerobic Gram-positive bacteria. The agent has been evaluated in two Phase 3 complicated skin and skin structure infection clinical trials.
Methods: Non-duplicate clinical isolates (919 total; see Table) of EF (579), EFM (318) and EAV (12) were submitted from 26 medical centres in Europe participating in TLV surveillance. Identifications were confirmed by the central monitor and all isolates were susceptibility tested using CLSI broth microdilution methods.
Results: Among the comparators, TLV was the most potent agent tested against *Enterococcus* spp. (EF and EFM; MIC50 values, 0.25 and 0.06 mg/L, respectively; see Table) compared with vancomycin (VAN; 1 and 1 mg/L), daptomycin (1 and 2 mg/L), levofloxacin (1 and >4 mg/L), and linezolid (1 and 1 mg/L). TLV was 4-fold more active (MIC50) than VAN against EF and 16-fold more active against EFM (only 15.4% of EFM had TLV MIC values of >1 mg/L compared with 29.6% having VAN MIC values >4 mg/L). Overall, 9.7% of tested enterococci were VAN-resistant, including 1.0% of EF and 25.8% of EFM; TLV remained ⩾16-fold more potent (MIC50) than VAN against these resistant EFM strains. Among the comparators, only daptomycin and linezolid were uniformly active against all enterococci (>99% susceptible), followed by teicoplanin (92.4%) and VAN (88.9%). All but one strain of EAV were inhibited by ⩽0.06 mg/L of TLV.

Table. Antimicrobial activity of telavancin against year 2007 enterococcal isolates

Organism (N)	MIC (mg/L)		Cumulative % inhibited at MIC (mg/L)				
	50%	90%	⩽0.06	0.12	0.25	0.5	1
EF (579)	0.25	0.5	1	22	84	99	99
VAN-susceptible (573)	0.25	0.5	1	23	84	100	–
VAN-resistant (6)	>2	>2	0	0	0	0	0
EFM (318)	0.06	2	66	79	80	80	85
VAN-susceptible (224)	0.06	0.12	82	99	100	–	–
VAN-resistant (82)	2	>2	18	21	21	22	40
EAV (12)	0.06	0.06	92	92	92	92	100

EAV, *Enterococcus avium*; EF, *E. faecalis*; EFM, *E. faecium*; VAN, vancomycin.

Conclusions: Based on MIC50 potencies, TLV was the most active agent tested against European (2007) *Enterococcus* spp. isolates, and inhibited 94.0% of strains at ⩽1 mg/L, whereas only 88.9% were inhibited by ⩽ 4 mg/L of VAN (current breakpoint). Continued monitoring for resistance emergence in enterococci and other Gram-positive pathogens will be critical in assessing the long-term efficacy of this promising agent.

P533 Activity of telavancin tested against *viridans* group and beta-haemolytic streptococci, and multidrug-resistant *Streptococcus pneumoniae*

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Objectives: To evaluate the activity of telavancin (TLV) tested against *viridans* group streptococci (VGS, five species), beta-haemolytic streptococci (BHS) and *Streptococcus pneumoniae* (SPN), including multidrug-resistant strains. TLV is a novel, rapidly bactericidal lipoglycopeptide active against Gram-positive pathogens, including resistant subsets, and has been evaluated in complicated skin and skin structure infection clinical trials.
Methods: The activity of TLV was compared with those of other antimicrobial classes using reference broth microdilution (CLSI, M7-A7; Mueller-Hinton broth supplemented with 2–5% lysed horse blood) methods tested against 1005 streptococci (100 each of five VGS species; 100 each of Lancefield Groups B, C and G BHS; and 205 SPN, including multidrug-resistant patterns [>3 classes]) recovered from global surveillance programmes.
Results: Among tested VGS, 99.8% had TLV MICs of ⩽0.06 mg/L. All MIC50 and MIC90 values for *S. anginosus*, *S. constellatus*, *S. mitis* and *S. oralis* were 0.03 mg/L; *S. intermedius* showed an elevated MIC90 (0.06 mg/L) and the highest TLV MIC (0.25 mg/L). Serogroups B, C and G BHS had the same modal TLV MIC (0.03 mg/L), but MIC90 results varied from 0.03 mg/L for Group C BHS to 0.06 mg/L for Groups B and G BHS. All BHS were inhibited by ⩽0.12 mg/L. Against SPN, TLV had a pronounced modal MIC at 0.015 mg/L (also MIC50 and MIC90) and 99.5% of results were either 0.008 or 0.015 mg/L (highest MIC). No difference in the TLV MIC90 (0.015 mg/L) was observed, but the MIC50 was slightly lower (0.008 mg/L) for two resistance phenotypes (penicillin-nonsusceptible [30 strains] and erythromycin-resistant [10 strains]). TLV MIC results did not correlate with mechanisms of resistance found for β-lactams, macrolides, tetracyclines, fluoroquinolones and trimethoprim/sulfamethoxazole. Overall, >99% of TLV MIC results occurred within three dilution steps (0.015–0.06 mg/L) for the tested streptococci.
Conclusions: TLV was found to be highly potent against prevalent VGS, BHS and SPN. All isolates were inhibited by ⩽0.06 mg/L TLV, with two exceptions (0.2%): one group G BHS at 0.12 mg/L and one *S. intermedius* at 0.25 mg/L. These results demonstrate that TLV may be an excellent therapeutic candidate for serious infections caused by these pathogenic organisms.

P534 Antimicrobial activity of telavancin and comparator agents tested against recently isolated (2007) European *Staphylococcus aureus* and coagulase-negative staphylococci

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Objectives: Telavancin (TLV) is an investigational, novel, rapidly bactericidal lipoglycopeptide that is broadly active against Gram-positive pathogens, and has completed complicated skin and skin structure infection clinical trials. Given concerns over the rapid emergence of resistance among staphylococci, including community-acquired strains, we compared the potency of TLV versus other antimicrobials against contemporary oxacillin-susceptible (OX-S) and OX-resistant (OX-R) *Staphylococcus aureus* (SA) and coagulase-negative staphylococci (CoNS) collected as part of a European antimicrobial resistance surveillance programme.
Methods: Consecutive, non-duplicate patient isolates (n = 2834) were submitted from 26 medical centres in Europe (10 countries), Turkey and

Israel during 2007 (2202 SA [OX-R, 29.3%], 632 CoNS [OX-R, 76.1%]) and susceptibility tested using CLSI (M7-A7) broth microdilution methods.

Results: Compared with OX-S SA, TLV MIC90 values varied by one dilution in OX-R SA (0.12 versus 0.25 mg/L, respectively; see Table), but was unchanged for OX-R CoNS (0.25 mg/L); all isolates were inhibited by ⩽0.5 mg/L. TLV was 2-, 4- and 8-fold more potent (MIC90) than daptomycin, vancomycin and linezolid, respectively, when testing SA, and 2-, 8- and 4-fold more potent, respectively, when testing CoNS. Among CoNS, TLV was most active against *S. lugdunensis* (MIC50, 0.06 mg/L) and least active against *S. warneri* (MIC50, 0.25 mg/L; 10 isolates each); MIC50 values for other species (*S. capitis* [20 isolates], *S. epidermidis* [316 isolates], *S. haemolyticus* [34 isolates], and *S. hominis* [59 isolates]) were all 0.12 mg/L. High levels of R to other agents were observed among OX-R SA and CoNS with respective R rates (%) as follows: erythromycin (69.8/68.0), clindamycin (30.0/29.7), gentamicin (19.7/37.9), levofloxacin (90.7/65.7), tetracycline (11.6/18.3) and trimethoprim/sulfamethoxazole (1.9/45.3).

Organism (N)	MIC (mg L)							
	TLV		VAN		LEV		LZD	
	50%	90%	50%	90%	50%	90%	50%	90%
OX-S SA (1556)	0.12	0.12	1	1	⩽0.5	⩽0.5	1	2
OX-R SA (646)	0.12	0.25	1	1	>4	>4	1	2
OX-S CoNS (151)	0.12	0.25	1	2	⩽0.5	⩽0.5	0.5	1
OX-R CoNS (481)	0.12	0.25	2	2	4	>4	1	1

LEV, levofloxacin; LZD, linezolid; TLV, telavancin; VAN, vancomycin.

Conclusions: TLV displayed higher potency than the other agents tested against SA and CoNS (MIC50 and MIC90 values for both, 0.12 and 0.25 mg/L), and inhibited all isolates at ⩽0.5 mg/L. TLV exhibited similar potency for OX-S and -R strains. The continued and rapid emergence of resistant staphylococci, including community-associated OX-R SA, necessitates the timely introduction of new therapeutic agents and longitudinal surveillance to assist in control efforts.

P535 Activity of telavancin against isolates from recently completed Phase 3 studies of complicated skin and skin structure infections

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Objective: Telavancin (TLV) is a novel, bactericidal lipoglycopeptide with a broad and potent Gram-positive spectrum of activity that includes meticillin-resistant *Staphylococcus aureus* (MRSA). TLV possesses a unique, multifunctional mechanism of action that includes inhibition of cell wall biosynthesis and disruption of bacterial membrane function. The efficacy and safety of TLV have been studied in patients with complicated skin and skin structure infections (cSSSIs) in two methodologically-identical, worldwide, Phase 3 studies (ATLAS 1 and ATLAS 2). Here, we report the susceptibility testing results for TLV and comparators against the Gram-positive isolates collected in these studies.

Methods: A total of 1506 baseline Gram-positive isolates were obtained from 1867 cSSSI patients enrolled in the ATLAS studies throughout North America, Europe, South Africa, South America and Asia during 2005 and 2006. All isolates were identified and susceptibility tests were performed at a central laboratory. MIC values were determined by the CLSI broth microdilution method, using frozen reference MIC panels.

Results: MIC50, MIC90 and ranges for TLV against staphylococci, streptococci and enterococci are shown in the table. Based on MIC90 comparisons, TLV was 2–4-fold more potent than vancomycin, and up to 2-fold more potent than teicoplanin against staphylococci. Teicoplanin was more active against enterococci (MIC90 = 0.25 microg/mL) than TLV or vancomycin (MIC90 = 1 microg/mL and 2 microg/mL, respectively), but less active against coagulase-negative staphylococci (MIC50 = 2 microg/mL). Daptomycin MIC values were similar to TLV against staphylococci and *S. pyogenes*, but were elevated for *S. agalactiae* (MIC90 = 0.25 microg/mL), other *Streptococcus* spp. (MIC90 = 1 microg/mL) and vancomycin-resistant enterococci (MIC90 = 2 microg/mL for *E. faecalis* and MIC50 = 2 microg/mL for *E. faecium*/*E. avium*, respectively). Linezolid MIC90 values were consistently 1–4 microg/mL.

Organism	No. of isolates	TLV MIC (μg/mL)		
		MIC range	MIC_{50}	MIC_{90}
Staphylococcus aureus	1214	0.12–1	0.5	0.5
MSSA	464	0.12–1	0.5	0.5
MRSA	750	0.12–1	0.5	0.5
CoNS	6	0.25–0.5	0.5	NA
Streptococci	201	0.015–0.12	0.06	0.06
S. pyogenes	61	0.015–0.12	0.03	0.06
S. agalactiae	46	0.06–0.12	0.06	0.06
Others*	94	0.015–0.12	0.06	0.06
Enterococci	85	0.06–1	0.5	1
E. faecalis	78	0.25–1	0.5	1
E. faecium and *E. avium*	7	0.06–0.25	0.12	NA

CoNS, coagulase-negative staphylococci; MRSA: meticillin-resistant *S. aureus*; MSSA, meticillin-susceptible *S. aureus*.
*Includes *S. acidominimus*, *S. anginosus*, *S. bovis*, *S. canis*, *S. constellatus*, *S. dysgalactiae*, *S. dysgalactiae ssp. equisimilis*, *S. intermedius*, *S. mitis*, *S. oralis*, *S. pneumoniae* and *viridans* group streptococci.

Conclusions: TLV was active against Gram-positive pathogens common in cSSSIs. Based upon MIC90, TLV was among the most active agents tested. These data highlight the potential therapeutic use of TLV in the treatment of cSSSIs due to Gram-positive pathogens.

P536 Activity of telavancin against complicated skin and skin structure infection isolates according to specimen source

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Objective: Telavancin (TLV) is a novel, bactericidal lipoglycopeptide with a broad and potent Gram-positive spectrum of activity. TLV's unique, multifunctional mechanism of action includes inhibition of bacterial cell wall biosynthesis and disruption of bacterial membrane function. The efficacy and safety of TLV were studied in patients with complicated skin and skin structure infections (cSSSIs) in two identical, worldwide, Phase 3 studies (ATLAS 1 and ATLAS 2). Here, we report the susceptibility testing results by infection type (major abscess, deep/extensive cellulitis, wound, burn or ulcer) for TLV against the Gram-positive isolates collected in these studies.

Methods: A total of 1506 baseline Gram-positive isolates were obtained from among the 1867 cSSSI patients enrolled in the ATLAS studies throughout North America, Europe, South Africa, South America and Asia during 2005–06. All isolates were identified and susceptibility tests were performed at a central laboratory. MIC values were determined by the CLSI broth microdilution method, using frozen reference MIC panels.

Results: See Table.

Conclusions: TLV was active against Gram-positive pathogens common in cSSSIs, irrespective of the infection site. These data highlight the potential therapeutic use of TLV in the treatment of Gram-positive cSSSIs at a variety of infection sites.

Organism	No. of isolates	TLV MIC (μg/mL)		
		MIC range	MIC_{50}	MIC_{90}
Staphylococci (1220)				
Major abscess	638	0.25–1	0.5	0.5
Deep/extensive cellulitis	303	0.12–1	0.25	0.5
Wound infection	191	0.12–1	0.5	0.5
Infected burn	16	0.25–0.5	0.25	0.5
Infected ulcer	72	0.12–1	0.5	0.5
Streptococci (201)				
Major abscess	91	0.015–0.12	0.06	0.06
Deep/extensive cellulitis	68	0.015–0.06	0.03	0.06
Wound infection	22	0.015–0.12	0.015	0.03
Infected burn	5	0.015–0.06	0.03	NA
Infected ulcer	15	0.015–0.12	0.06	0.12
Enterococci (85)				
Major abscess	19	0.25–1	0.5	1
Deep/extensive cellulitis	30	0.25–1	0.5	1
Wound infection	14	0.25–1	0.5	1
Infected burn	2	0.5	NA	NA
Infected ulcer	20	0.06–1	0.5	1

P537 Cell wall and membrane effects of oritavancin on *Staphylococcus aureus* and *Enterococcus faecalis*

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Objective: The lipoglycopeptide oritavancin (ORI) exerts rapid bactericidal activity in vitro against meticillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecalis* (VRE), distinguishing it from glycopeptides like vancomycin (VAN). In this study, we compared the ultrastructural effects of ORI and VAN on MRSA and of ORI on VRE by transmission electron microscopy (TEM).
Methods: Exponentially-growing *S. aureus* ATCC 43300 (MRSA, 10^9 CFU) was exposed to 1 mg/L ORI or 16 mg/L VAN (16 X their respective broth microdilution minimal inhibitory concentrations) for 10 min whereas the VRE clinical isolate *E. faecalis* A5241515 was exposed to ORI at 1 mg/L (16 X its broth microdilution minimal inhibitory concentration) for 10 min. Bacteria were fixed in 2.5% glutaraldehyde, embedded and thin sectioned for TEM. TEM was performed using a Philips CM10 under standard operating conditions at 100 kV.
Results: Compared to VAN treatment, fewer cells were present in ORI-exposed MRSA samples, an indication that some cell lysis had occurred. The presence of cell ghosts (remnants of fixed lysed cells) indicated that lysis occurred mainly at the cell wall peripheral to the septum. Both ORI and VAN induced membrane inclusions; however, the effects of ORI were noticeably more pronounced. ORI had a distinct effect on the septum as evidenced by deformations and loss of the 'mid-line', a region of dense staining due to the activity of autolysins that cleave cell wall polymers during daughter cell separation. Interestingly, ORI did not affect the mid-line of the VRE isolate. VRE exposed to ORI had small, thin fibers protruding from division scars, the visible junction between existing and nascent cell wall in enterococci. These fibers probably resulted from cell wall breakdown as examination of VRE cell ghosts showed that rupture occurred at these sites. ORI-exposed VRE also exhibited membrane inclusions and septal distortions.
Conclusion: TEM data strongly suggest that ORI affected the coordination of cell division in *S. aureus*. ORI apparently induced breakdown of the cell wall at division scars in VRE. These effects on MRSA and VRE suggest that ORI may act largely during growth and division of these important pathogens.

P538 Anti-enterococcal and anti-sreptococcal activity profile of oritavancin in Europe

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Objective: Oritavancin (ORI) is a bactericidal lipoglycopeptide under clinical development for the treatment of infections caused by a variety of Gram-positive organisms (Org). Vancomycin (VAN)-resistant enterococci (VRE), in particular *E. faecium* (EM) with the vanA genotype isolated from non-hospitalised persons, are prevalent in Europe (EU). This surveillance initiative established a current in vitro activity profile of ORI against enterococci (EN) and streptococci (STR), including those resistant (R) to currently available agents.
Methods: Recent (2005) clinical isolates of *E. faecalis* (EF; n = 151), EM (n = 150), *S. pneumoniae* (SP; n = 100) and b-hemolytic streptococci ([BS]; 41 *S. pyogenes* [SPY], 26 *S. agalactiae* [SAG], 11 Group C, G and F streptococci) collected from 35 hospital sites across 14 countries in EU, were centrally tested by broth microdilution (CLSI; M7-A7). ORI assays included 0.002% polysorbate-80 throughout. ORI activity for EN was analysed according to VAN susceptibility (S) and for STR according to macrolide or penicillin (PEN) S status. Additionally, multi-drug resistance [MDR; concurrent resistance to $\geqslant$ 2 of the agents listed: PEN, cefuroxime, erythromycin (ERY), and trimethoprim-sulfamethoxazole] among SP was examined.
Results: See table.

Org	Category	N	ORI mg/L	
			MIC range	MIC_{90}
EF	All	151	⩽0.0005–1	0.25
	VAN S	132	⩽0.0005–0.5	0.06
	VAN NS	19	0.03–1	1
EM	All	150	0.008–0.5	0.12
	VAN S	117	0.008–0.25	0.03
	VAN NS	33	0.008–0.5	0.5
SPY	All	41	0.015–0.5	0.25
	ERY S	34	0.015–0.5	0.25
	ERY NS	7	0.03–0.12	NA
BS	All	11	0.001–0.12	0.12
	ERY S	7	0.001–0.12	NA
	ERY NS	4	0.03–0.12	NA
SP	All	100	⩽0.0005–0.015	0.008
	PEN S	67	⩽0.0005–0.015	0.008
	PEN I	18	0.001–0.008	0.008
	PEN R	15	0.002–0.015	0.008
	MDR	26	0.002–0.015	0.008
	Non-MDR	74	⩽0.0005–0.015	0.008
SAG	All	26	0.03–0.25	0.25
	ERY S	20	0.03–0.25	0.25
	ERY NS	6	0.06–0.25	NA

NA = not applicable for <10 isolates; NS = non-susceptible (intermediate and R).

Conclusions: ORI showed potent activity against all EN; however, the ORI MIC90 was higher for VAN NS versus VAN S enterococci. Based on MIC90, ORI was active against all STR, regardless of resistance phenotype. Based on these findings, ORI offers promise for the treatment of infections caused by EN or STR, including those R to currently used agents.

P539 In vitro activity profile of oritavancin against resistant staphylococcal populations from a recent surveillance initiative in Europe

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Objectives: Oritavancin (ORI), currently in clinical development for use in the treatment of infections caused by Gram-positive bacteria, is a potent bactericidal lipoglycopeptide. ORI has previously shown potent activity against staphylococci (STA), including resistant (R) strains. This surveillance (SUR) initiative was undertaken to profile the in vitro activity of ORI against drug-R and multi-drug resistant (MDR) STA in Europe (EU).

Methods: Recent (2005–2006) clinical isolates of *S. aureus* (SA; n = 557) and coagulase-negative staphylococci (CoNS; n = 78) from hospital sites in EU (48 sites; 15 countries), were centrally tested by broth microdilution (CLSI; M7-A7) against ORI and relevant comparators. ORI assays included 0.002% polysorbate-80 throughout. ORI activity was analysed for STA according to oxacillin (OX) susceptible (S) status and MDR phenotypes. MDR was defined as concurrent R to ⩾3 of the following agents: ciprofloxacin (CIP), clindamycin (CLI), erythromycin (ERY), gentamicin (GEN), Ox, quinupristin-dalfopristin, trimethoprim-sulfamethoxazole (SXT), vancomycin, daptomycin (non-susceptible [NS]), and linezolid (NS).

Results: 58.9% of SA and 61.5% of CoNS had an MDR phenotype. The most prevalent MDR phenotypes among SA included 16.7% CIP-R, ERY-R, and OX-R; and 16% CIP-R, ERY-R, OX-R, and CLI-R. Among CNS, 24.4% were 6-drug R which included CIP, CLI, ERY, GEN, OX, and SXT.

Organism	Phenotype	N	ORI mg/L	
			MIC range	MIC_{90}
SA	All	557	0.008–4	0.25
	Ox S	152	0.008–0.25	0.12
	Ox R	405	0.015–4	0.25
	MDR	328	0.008–4	0.25
	Non-MDR	229	0.015–0.25	0.25
CoNS	All	78	0.008–1	0.25
	Ox S	22	0.008–1	0.25
	Ox R	56	0.015–1	0.25
	MDR	48	0.015–1	0.25
	Non-MDR	30	0.008–1	0.25

Conclusions: SUR studies are essential for reviewing current trends in in vitro susceptibility patterns among antimicrobial agents used to treat serious infections. ORI had potent and consistent activity against a diverse EU collection of staphylococci which included OX-R and MDR phenotypes.

P540 Comparison of oritavancin versus vancomycin as treatment for clindamycin-induced *C. difficile* ribotype 027 infection in a human gut model

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Objectives: *C. difficile* infection (CDI) is a nosocomial disease of increasing importance. First line treatment is limited currently to metronidazole or vancomycin (VAN). Recent reports have questioned the efficacy of current therapies for CDI, particularly against an apparently hypervirulent *C. difficile* (CD) strain, PCR ribotype 027 (NAP1/BI). Oritavancin (ORI) is a lipoglycopeptide with activity against Gram-positive bacteria, including drug-resistant pathogens. We evaluated the efficacy of ORI and VAN in treatment of clindamycin-induced CDI in separate experiments in an in vitro human gut model.

Methods: CD PCR ribotype 027 was used in both experiments. Clindamycin instillation was used to achieve human gut concentrations and to induce CD spore germination, proliferation and high-level cytotoxin production. ORI and VAN were instilled at levels equivalent to expected human faecal concentrations. Gut flora and CD (vegetative cells and spores) were recovered and enumerated using selective media and viable counting. CD toxin was detected by cytotoxin assay.

Results: Clindamycin exposure elicited CD proliferation and high-level (5RU) cytotoxin production in both experiments on day 35. ORI and VAN levels peaked at 52 and 1144 mg/L in vessel 3, respectively. VAN instillation reduced vegetative CD counts within 1 day but did not affect CD spore counts. On day 2 of ORI instillation both CD total and spore counts declined by approx 2 logs cfu/mL; on day 3 counts were below the limits of detection and remained so during the rest of ORI instillation. Centrifugation and washing of culture samples, in addition to exposure to activated charcoal (20–40g/L) did not enhance recovery of *C. difficile*. Cytotoxin titres declined to the limits of detection in both experiments but did so 5 days sooner in the VAN experiment. Further CD proliferation and high-level cytotoxin production occurred 12 days after the end of VAN instillation, whereas CD in the ORI experiment was only sporadically isolated at the limits of detection.

Conclusion: Both ORI and VAN were effective in treating clindamycin-induced CDI in a human gut model, but only ORI appeared active against spore forms of CD. Recurrence of toxin production was observed following VAN instillation but not ORI. These results suggest that ORI should be as effective as VAN in treating CDI, and may prevent recrudescence of CD spores.

P541 Dalbavancin (DECIDE) tested against indicated Gram-positive species in European medical centres: results from France

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Objective: To assess the dalbavancin (DAL) spectrum and potency when tested against recent clinical isolates from five medical centres in France from October to December, 2007 (374 strains).

Methods: Standardised and reference-quality susceptibility (S) methods of agar diffusion were applied by each investigator. Etest (ET; AB BIODISK, Solna, Sweden) and CLSI (M2-A9) disk diffusion (DD) tests were performed with concurrent quality control (CLSI M100-S17, 2007) and repeated testing of strains showing unusual resistance (R) patterns in linezolid (LZD)-R, vancomycin (VAN) or DAL-non-S. A total of 374 strains were tested against DAL and VAN by ET; and LZD, cefoxitin (meticillin susceptibility surrogate), levofloxacin (LEV), gentamicin (GEN), tetracycline (TC), erythromycin (ERY), clindamycin (CC; plus D-test, penicillin (PEN) and ceftriaxone (CRO) by DD. All five French sites had acceptable quality control results. DAL-S was defined at ⩽0.25 mg/L for staphylococci and streptococci.

Results: DAL exhibited ⩾16-fold greater activity than VAN against the 251 *S. aureus* (MIC50/90, 0.094/0.125 mg/L), 50 coagulase-negative staphylococci (CoNS; MIC50/90, 0.064/0.125 mg/L) and 73 beta-haemolytic streptococci (BHS; MIC50/90, ⩽0.016/0.032 mg/L). The most common beta-streptococci group was *S. pyogenes* (approx. 60% of strains). MRSA rates were uniform from 20 to 29% (average at 25%) among sites and S rates across all staphylococci were LZD (100%), LEV (55–74%), ERY (59–73%), CC (80–86% with additional 9–18% inducible R), TC (93–94%) and GEN (63–98%). D-test positive rates in BHS and CoNS were 0 and 47% of ERY-R/CC-S isolates, respectively. LEV-R BHS (one each of gr. A and G) were detected in Paris. The most elevated DAL MIC results were 0.25 mg/L (two strains, SA and CoNS) from Paris and Lyon.

Conclusions: DAL, a novel glycolipopeptide (t1/2 at 6–8 days; once weekly dosing) showed high activity (MIC90 range, ⩽0.032–0.125 mg/L) against tested staphylococci and BST from France. The documented DAL activity (⩾16-fold greater than VAN) exhibited wide

spectrum of coverage of contemporary Gram-positive pathogens endemic to the five centres including strains R to other antimicrobial classes.

Table: Comparative activity of DAL in France (5 sites)

Method/Antimicrobial	% S activity by pathogen (no.)		
	S. aureus (251)	CoNS (50)	BHS (73)
Etest			
Dalbavancin	100	100	100
Vancomycin	100	100	100
Disk diffusion			
Linezolid	100	100	99
Erythromycin	73	59	73
Clindamycin[a]	86 (9)	80 (18)	73 (0)
Levofloxacin	74	55	94
Oxacillin[b]	75	49	NT

a. Includes % R results with D-test inducible CC-R in parentheses.
b. MRSA rate was 25% tested by cefoxitin disks (⩽21 mm); and all BHS were CRO- and PEN-S.

P542 Comparative activity of dalbavancin tested against indicated Gram-positive species in Europe: results from two Spanish medical centres (DECIDE Program)

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Objective: To assess the activity of dalbavancin (DAL) tested against recent clinical Gram-positive isolates from two medical centres in Spain over the time interval of October to December, 2007. DAL has documented activity comparable to glycopeptides (vancomycin [VAN] and teicoplanin), a prolonged elimination half-life and clinical success in trials for complicated skin and skin structure infections. Potency of DAL was determined separately in five European nations to monitor potential geographic variations against indicated species.

Methods: Standardised and reference quality susceptibility (S) methods of agar diffusion were applied by each investigator. Etest (ET; AB BIODISK, Solna, Sweden) and CLSI (M2-A9) disk diffusion (DD) tests were performed with concurrent quality control (CLSI M100-S17, 2007) and repeated testing of strains showing unusual resistance (R) patterns of linezolid (LZD)-R, and VAN or DAL-non-S. A total of 150 strains were tested against DAL and VAN by ET, and LZD, cefoxitin (for meticillin testing), levofloxacin (LEV), gentamicin (GEN), tetracycline (TC), erythromycin (ERY), clindamycin (CC; plus D-test), penicillin (PEN) and ceftriaxone (CRO) by DD. All sites had acceptable control results using three QC strains. DAL-S was defined as ⩽0.25 mg/L.

Results: DAL exhibited high potency against the 100 *S. aureus* (MIC50/90, 0.047/0.094 mg/L), coagulase-negative staphylococci (CoNS; MIC50/90, 0.047/0.125 mg/L) and beta-haemolytic streptococci (BHS; MIC50/90, ⩽0.016/⩽0.016 mg/L). This activity (MIC50 comparison) was 16-, 32- and 64-fold greater than VAN, respectively. MRSA rates varied from 6 to 24 (15% overall) between sites and *S. aureus* S rates were LZD (100%), LEV (82%), ERY (75%), CC (96% with additional 9% inducible R). D-test positive rates in CoNS were 5%; overall CC-R was at 35%. Meticillin R did not adversely influence DAL activity. All BHS (63% *S. pyogenes*) were PEN- and CRO-S. GEN (75–95% S) and TC (85–96% S) were modestly active versus the *S. aureus* and CoNS strains.

Conclusions: DAL, a novel long-acting glycolipopeptide (once weekly dosing) demonstrated high activity (MIC90 ranges, ⩽0.016–0.125 mg/L) against tested staphylococci and BHS from Spanish hospitals. The highest recorded MIC was 0.25 mg/L, confirmed in a CoNS from Madrid. The exhibited DAL potency (⩾16-fold greater than VAN) appears to cover contemporary Gram-positive pathogens endemic in this area of Europe.

Comparative activity of DAL in Spain

Antimicrobia1	% S activity by pathogen (no.)		
	S. aureus (100)[a]	CoNS (20)	BHS (30)[b]
Dalbavancin	100	100	100
Vancomycin	100	100	100
Linezolid	100	100	100
Erythromycin	75	50	87
Clindamycin[c]	87	65	90
Levofloxacin	82	50	90
Ceftriaxone	NT	NT	100

a. MRSA rate was 15%.
b. Includes gr. A (19), gr. B (3), gr. C (2), gr. F (2) and gr. G (4), and all BHS were PEN-S (zone, ⩾29 mm).
c. Includes results from D-test (inducible CC-R).

P543 Daptomycin susceptibility testing of linezolid-resistant *Staphylococcus cohnii* isolates

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Objectives: Daptomycin is a cyclic lipopeptide with potent activity and broad spectrum against Gram-positive bacteria approved for the treatment of complicated skin and skin structure infections and bacteraemia, including right sided endocarditis. The aim of this study was to evaluate the susceptibility of daptomycin against 26 clinical isolates of multi-drug resistant *Staphylococcus cohnii*, all being resistant to linezolid, recovered from blood cultures.

Methods: 26 non-duplicate isolates of *Staphylococcus cohnii* were tested for susceptibility to daptomycin with the method of E-test (AB Biodisk, Solna, Sweden) according to the manufacturer's recommendations. Those isolates were isolated from blood cultures of patients hospitalised in KAT hospital during a period of approximately three years (July 2005 to November 2007). Susceptibility testing was performed using the VITEK 2 System (Biomerieux, France). MICs for linezolid were also determined with the use of E-test. Interpretation of MICs was according to CLSI guidelines.

Results: All isolates were found resistant to oxacillin, ciprofloxacin, clindamycin, erythromycin, quinupristin/dalfopristin and susceptible to gentamicin, teicoplanin, tetracyclin, trimethoprim/sulfamethoxazole, and vancomycin. Susceptibility to fucidic acid and rifampicin varied from intermediate to resistant. Most isolates were intermediately resistant to moxifloxacin except for two that were resistant. All isolates were resistant to linezolid (MIC ⩾8 mg/L) and susceptible to daptomycin with MICs ranging between 0.5 mg/L (8 isolates, 30.8%), to 0.75 mg/L (11 isolates, 42.3%) and up to 1.0 mg/L (7 isolates, 26.9%).

Conclusion: Daptomycin showed excellent in vitro activity against multi-drug resistant isolates of *Staphylococcus cohnii*. It seems to be a promising solution to such isolates of Gram-positive bacteria while other therapeutic options such as linezolid, teicoplanin and quinupristin/dalfopristin are being eliminated by increasing resistance.

P544 In vitro time-kill studies of oritavancin against drug-resistant isolates of *Staphylococcus aureus*

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Background: Oritavancin (ORI), a semi-synthetic lipoglycopeptide, exerts bactericidal activity against Gram-positive bacteria including vancomycin (VAN)-resistant *S. aureus* and enterococci. To characterise ORI activity in vitro we performed time-kill (TK) experiments against *S. aureus* of clinical importance, including recent antibiotic-resistant isolates.

Methods: 6 strains of *S. aureus* (1 meticillin-resistant [MRSA], 1 community-acquired [CA] MRSA, 1 linezolid-resistant MRSA, 1 heterogeneous VAN-intermediate [hVISA], and 2 VAN-resistant [VRSA]) were tested in TK assays based on CLSI guidelines. ORI assays included 0.002% polysorbate-80 throughout. ORI and comparators VAN, teicoplanin (TEI), linezolid (LIN) and daptomycin (DAP) were tested at static concentrations approximating their free peak (fCmax) and free trough in plasma when administered at standard doses. Cell counts were determined by serial dilution plating.
Results: ORI showed concentration-dependent killing of all strains tested: at its fCmax (predicted from a 200 mg dose in humans), ORI displayed bactericidal activity (⩾ 3 log kill relative to starting inoculum) against the MRSA, CA MRSA and linezolid-resistant MRSA within 2 h. Against VRSA, ORI demonstrated 3 log kill relative to starting inoculum within 3 h for VRS2 and 6 h for VRS1. At fCmax, ORI was bactericidal against hVISA at 24 h; at fCmax predicted from an 800 mg dose, ORI achieved 3 log kill by 6 h for this strain.
Conclusions: ORI displayed concentration-dependent killing of MRSA, VRSA, hVISA in vitro. ORI was more rapidly bactericidal against all bacteria tested than were VAN, TEI, LIN or DAP at physiologically-relevant concentrations. These data support the conclusion that ORI displays concentration-dependent bactericidal activity on recent, drug-resistant isolates of *S. aureus*.

P545 Clinical efficacy of iclaprim in complicated skin and skin structure infection: preliminary results from the ASSIST-2 clinical trial

M. Dryden, O. O'Hare, E. Sidarous, P. Hadvary, K. Islam (Winchester, Bournemouth, UK; Reinach, CH)

Objective: To compare the clinical efficacy of iclaprim (ICL) with that of linezolid (LZD) at the test-of-cure (TOC) visit in the ASSIST-2 (Arpida's Skin and Skin Structure Infection Study-2), randomised, multicentre, double-blind, Phase III clinical trial.
Methods: Patients with complicated skin and skin structure infection (cSSSI) were randomised to one of two treatment arms: intravenous ICL 0.8 mg/kg q 12 hours or intravenous linezolid (LZD) 600 mg q 12 hours and received 10–14 days treatment. Clinical cure rate at the TOC visit in the intent-to-treat (ITT) and the per protocol (PP) populations was the primary efficacy endpoint for this study. The non-inferiority margin assigned was <12.5% for the 95% confidence interval (CI) for treatment difference.
Results: The ITT population comprised 494 patients (ICL: 251; LZD: 243), with 404 of these patients eligible for the per-protocol (PP) population (ICL: 205; LZD: 195). The distribution of cSSSIs at baseline was: wound infection (45%), major abscess (30%), cellulitis (28%), infected ulcers (8%), and infected burns (7%), with little variation between the treatment groups. The most commonly isolated baseline pathogen was *Staphylococcus aureus* (309 isolates), accounting for 63% of all Gram-positive pathogens (ICL: 59%; LZD: 66%). Meticillin-resistant *S. aureus* (MRSA) represented 50% of all *S. aureus* strains (ICL: 50%; LZD: 50%). Clinical cure rates were comparable between treatment groups in the ITT population (ICL: 81.3%, 95% CI = 75.9%-85.9%; LZD: 81.9%, 95% CI = 76.5%-86.5%). In the PP population there was a small numerical difference between the two groups (ICL: 90.0%; LZD: 96.9%).However, the number of patients receiving prohibited antibiotics and being therefore excluded from the PP population was much higher in the linezolid group. In a clinically evaluable population including the patients taking prohibited antibacterial agents during the trial, the clinical cure rates were comparable (ICL: 84.7%, 95% CI = 79.3%-89.2%; LZD: 86.6%, 95% CI = 81.4%-90.9%). The 95% CI for treatment difference was –8.83% to 4.95% in the population.
Conclusions: ICL shows high efficacy in patients with cSSSI as evidenced by the high cure rates. ICL achieved the pre-specfified primary endpoint of non-inferior clinical cure rate when compared with LZD. ICL could be a useful addition to the antibacterial armamentarium for cSSSI.

P546 Dose linearity and gender-independent pharmacokinetics of intravenous iclaprim assessed in two randomised, double-blind and controlled cross-over studies

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Objectives: Iclaprim, a new antibiotic from the class of the diaminopyrimidines, had linear pharmacokinetics (PK) in the first-in-man dose escalation studies. In order to confirm the initial PK profile of iclaprim, two more PK studies were conducted.
Methods: In two randomised, double-blind, and placebo-controlled cross-over studies the PK profile in response to iclaprim dosing was investigated. In Study A, 24 healthy subjects (12 males [m], 12 females [f]) received a single intravenous (IV) infusion (150 mL in 30 minutes) of 1.6 mg/kg, 3.2 mg/kg or placebo applying a three-way cross-over design. In Study B, 24 healthy subjects (12 m, 12 f) received a single IV infusion (150 mL in 30 min) of 0.4 mg/kg, 0.8 mg/kg or 1.6 mg/kg (150 mL in 60 min) or matching placebo applying a four-way cross-over design. Blood samples for PK assessment were taken at pre-defined time points from pre-dose until + 24 hours post-administration (p.a.). Treatments were separated by wash-out phases.
Results: The mean age of the subjects in Study A was 31.1 yrs (range 19–45) and in Study B, it was 33.5 yrs (range 20–45). PK data obtained from these two studies are shown in the following table; SD values are shown in parentheses. PK parameters such as the elimination half-life, clearance and volume of distribution were comparable within the study groups (m vs. f) and also across the studies.

Dose	$AUC_{(0-inf)}$ (ng·h/mL)			C_{max} (ng/mL)		
	Overall	Male	Female	Overall	Male	Female
Study A						
1.6 mg/kg	4550 (1189)	4475 (977)	4619 (1395)	1519 (337)	1545 (232)	1495 (420)
3.2 mg/kg	9265 (2899)	9572 (2830)	8984 (3057)	2900 (553)	2921 (478)	2881 (635)
Study B						
0.4 mg/kg	1030 (281)	1003 (262)	1063 (313)	382 (79)	376 (91)	388 (66)
0.8 mg/kg	2082 (709)	2175 (712)	2004 (729)	831 (245)	866 (294)	801 (204)
1.6 mg/kg	4017 (1524)	4005 (1308)	4029 (1773)	1139 (290)	1109 (329)	1169 (256)

Conclusion: IV iclaprim PK after single doses (0.4 up to 3.2 mg/kg) exhibits dose linearity. The PK profile of iclaprim is independent of gender.

P547 Assessment of safety and tolerability profiles after iclaprim administration in complicated skin and skin structure infection from the ASSIST-2 clinical trial (Arpida's Skin and Skin Structure Infection Study-2)

P. Hadvary, M. Dryden, M. O'Hare, E. Sidarous, W. Danker, A. Leighton, K. Islam (Reinach, CH; Winchester, Bournemouth, UK; Durham, US)

Objective: To compare the safety and tolerability of iclaprim (ICL) with that of linezolid (LZD) in the ASSIST-2, randomised, multicentre, double-blind, Phase III clinical trial.
Methods: Patients with cSSSI were randomised to one of two treatment arms: intravenous ICL 0.8 mg/kg q 12 hours or 600 mg intravenous LZD q 12 hours and received 10–14 days treatment. The use of systemic or topical antibiotics, steroids or Type IA/III anti-arrhythmic drugs was prohibited during the study. Concomitant aztreonam and/or metronidazole were allowed.

Results: Safety and tolerability data were obtained from 494 patients in the ITT population (ICL: 250; LZD: 244). Treatment-emergent adverse events (AEs) were reported in 56% of patients (ICL 54%; LZD 59%). In general, ICL was better tolerated than LZD, with fewer patients reporting AEs in all categories (drug-related, severe, serious, and those that led to discontinuation of the patient from the study). The AEs were generally mild to moderate in intensity in both treatment arms. A listing of AEs that were possibly or probably related to study drug (>5%) is shown in the table.

Table: AEs possibly or probably related to study drug in >2% of patients

System organ class	ICL, n (%)	LZD, n (%)
Blood and lymphatic system disorders	0	7 (2.87%)
Gastrointestinal disorders	24 (9.60%)	38 (15.6%)
General disorders and administration site conditions	12 (4.80%)	10 (4.10%)
Infections and infestations	4 (1.60%)	7 (2.87%)
Investigations	31 (12.4%)	27 (11.1%)
Metabolism and nutrition disorders	3 (1.20%)	5 (2.05%)
Nervous system disorders	16 (6.40%)	26 (10.7%)
Psychiatric disorders	6 (2.40%)	1 (0.41%)
Respiratory and thoracic disorders	1 (0.40%)	8 (3.28%)
Skin and subcutaneous tissue disorders	13 (5.20%)	12 (4.92%)

Conclusions: ICL exhibits a good safety profile and was well tolerated in patients suffering from cSSSI. AEs of generally mild to moderate intensity were observed. These results indicate that iclaprim has an excellent safety and tolerability profile in patients with cSSSI.

P548 Analysis of the effect of iclaprim on the QT interval in the ASSIST-2 clinical trial

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Objective: To assess the effect of ICL on QT prolongation in the ASSIST-2 (Arpida's Skin and Skin Structure Infection Study-2), a randomised, multicentre, double-blind Phase III clinical trial.
Methods: Patients with cSSSI were randomised to one of two treatment arms: intravenous ICL 0.8 mg/kg q 12 hours or intravenous linezolid (LZD) 600 mg q 12 hours and received 10–14 days treatment. ECG monitoring was carried out as part of the safety evaluation of these patients and was performed at the following time points: Screening (within 1 day of study entry), Day 1 pre- (Baseline; BL) and post-dose, and Day 4 (post-dose).
Results: The ITT safety population comprised 494 patients (ICL: 250; LZD: 244). QT prolongation data corrected according to Bazett's formula (QTcB) are summarised in the Table.

Table: Mean value and mean change from BL in QTcB measurements

	ICL		LZD	
	Mean value (ms)	Mean change from BL (ms)	Mean value (ms)	Mean change from BL (ms)
BL (Day 1 pre-dose)	n = 249		n = 243	
mean (SD)	424.9 (24.7)	–	425.1 (23.8)	–
Day 1 post-dose	n = 243		n = 240	
mean (SD)	432.7 (27.1)	7.8 (13.7)	426.7 (23.8)	1.9 (11.9)
Day 4 post-dose	n = 240		n = 235	
mean (SD)	428.9 (26.5)	4.2 (18.0)	425.5 (24.8)	0.7 (17.4)

The mean QTcB change from BL on Day 1 (post-dose) was 7.8±13.7 ms and 1.9±11.9 ms in the ICL and LZD treatment groups, respectively. On Day 4 (post-dose), the mean change from BL was smaller; 4.2±18.0 ms (ICL) and 0.7± 17.4 ms (LZD). Three patients in the ICL treatment arm were reported to have median QTcB intervals of >500 ms after dosing on Day 1 and Day 4, compared with no patients in the LZD group. One patient and 0 patients in the ICL and LZD groups respectively had an increase of >60 ms from BL in QTcB interval on Day 1 post-dose, and no patients exhibited this increase on Day 4 post-dose. The percentage of cardiac adverse events possibly or probably related to study drug was higher in the LZD group (ICL: 0.68%, LZD: 1.4%), and no cardiac events were attributable to QTc prolongation.
Conclusions: Intravenous ICL tended to induce a small, transient prolongation of the mean QTc interval by approximately 4–6 ms post-dose. No cardiovascular adverse events related to QTc elevation were reported.

P549 Lack of effect of the novel antibiotic iclaprim on the anticoagulant activity and the pharmacokinetics of warfarin in healthy subjects

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Objectives: The aim of this study was to investigate the potential drug-drug interaction between the novel antibiotic iclaprim and the anticoagulant drug warfarin in healthy subjects.
Methods: This was an open-label, randomised, two-period cross-over study in twelve healthy male subjects. Administration of either a single oral dose of warfarin 25 mg alone (W) or a single oral dose of warfarin 25 mg on day three of an eight-day steady-state intravenous (IV) iclaprim treatment regimen (W+I) was performed. The treatment periods were separated by a washout period of at least 12 days. Plasma pharmacokinetics (PK) of R, S-warfarin and the anticoagulant activity of warfarin (INR – International Normalised Ratio) as well as PK of iclaprim were determined. Safety parameters of the warfarin and the iclaprim plus warfarin treatment regimens were assessed in addition.
Results: Total anticoagulant effect of warfarin was determined by calculation of the area under the effect vs. time curve; treatment ratio of W/W+I (AUEC: 1.059 [1.004, 1.116] 90% confidence interval) and the maximum INR observed (1.077 [1.020, 1.137]) were close to unity. Therefore, the anticoagulant activity of warfarin was not affected by concomitant treatment with iclaprim. Ratios for the area under the plasma concentration time curve (AUC0-infinity) of the more potent S-enantiomer of warfarin (1.009 [0.949, 1.074]) and the maximum plasma concentration (Cmax: 1.005 [0.947, 1.066]) were within the conventional bioequivalence ranges. Similar findings were obtained with regard to PK parameters of R-Warfarin which confirms lack of interaction between iclaprim and warfarin. Administration of warfarin during the course of iclaprim treatment had no effect on plasma PK of iclaprim determined after the first infusion as well as during steady-state of the antibiotic. Administrations of iclaprim and warfarin were well-tolerated by the study subjects.
Conclusions: Results from this study demonstrated that repeated intravenous infusions of iclaprim had no effect on the anticoagulant activity and plasma pharmacokinetics of orally administered warfarin. Thus, IV infusions of iclaprim may be administered to patients on warfarin treatment without the necessity for dose adjustments of the anticoagulant drug.

P550 Preliminary results of the microbiological efficacy of iclaprim in complicated skin and skin structure infection from the ASSIST-2 clinical trial

M. Jones, M. O'Hare, C. Burley, W. Danker, K. Islam, P. Hadvary (Reinach, CH; Bournemouth, UK; Durham, US)

Objective: To compare the clinical efficacy and microbiological cure rate of iclaprim (ICL) with that of linezolid (LZD) at the test-of-cure (TOC) visit in the ASSIST-2 (Arpida's Skin and Skin Structure Infection Study-2), randomised, multicentre, double-blind Phase III clinical trial.
Methods: Patients with cSSSI were randomised to one of two treatment arms: intravenous ICL 0.8 mg/kg q 12 hours or intravenous linezolid

(LZD) 600 mg q 12 hours and received 10–14 days treatment. By-patient, by-pathogen, and overall bacteriological response rates were secondary endpoints in this study.
Results: Of the 494 patients in the intent-to-treat (ITT) population, 376 patients had a Gram-positive pathogen isolated at baseline and were included in the modified-intent-to-treat (MITT) population (ICL: 192; LZD: 184). Gram-positive pathogens accounted for 72.3% of the total number of pathogens isolated at baseline, while *Staphylococcus aureus* (309 isolates) was the most commonly isolated pathogen at baseline from the Gram-positive group, accounting for 62.6% of all Gram-positive pathogens (ICL: 59.4%; LZD: 65.8%). Meticillin-resistant *S. aureus* (MRSA) made up almost half of all *S. aureus* strains (ICL: 49.7%; LZD: 50.0%). Clinical cure rates in both treatment arms in the MITT population were comparably high (ICL: 81.3%, LZD: 81.5%). The by-patient and by-pathogen bacteriological response for *S. aureus* (eradication/presumed eradication vs persistence) at the TOC visit are shown in the table below for the MITT population.

Bacteriological response	ICL		LZD	
	n	%	n	%
By patient	n = 192		n = 184	
Eradication	148	77	144	78
By pathogen				
S. aureus (total)	n = 149		n = 160	
Eradication	115	77	126	79
Persistence	21	14	12	8
MRSA	n = 74		n = 80	
Eradication	55	74	60	75
Persistence	10	14	8	10

Conclusions: ICL shows good clinical and microbiological efficacy against *S. aureus*, including MRSA, the major causative pathogen in cSSSI. These rates were comparable to those observed for LZD. ICL could constitute a useful antibacterial for the treatment of cSSSI including infections caused by MRSA.

P551 Pre-clinical studies of a new quinolone (UB-8902) against *Acinetobacter baumannii* resistant to ciprofloxacin

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Objectives: To evaluate the in vitro and in vivo activity of a new derivative of ciprofloxacin (UB-8902) against *Acinetobacter baumannii*.
Methods: We used two *A. baumannii* strains (Ab58 and Ab661). The antibiotics of the study were ciprofloxacin, moxifloxacin, and the new generated quinolone UB-8902. Animals used were immunocompetent C57BL/6 female mice.
In vitro tests: MIC (microdilution method) and bactericidal activity (time-kill curves) were performed following CLSI recomendations.
In vivo assays: we studied the toxicity parameters of the UB-8902 [lethal dose 0 (LD0), lethal dose 50 (LD50), and lethal dose 100 (LD100)] inoculating groups of 6 animals with rising concentrations in base 2 of UB-8902, from 0.5 mg/kg/ip to reach a 100% of mortality (Reed and Muench method). To ascertain the bacterial minimum lethal dose (MLD) and the bacterial lethal dose 50 (BLD50) of the strains Ab58 and Ab661, groups of 10 animals were inoculated (using a murine model of peritoneal sepsis) with a potentially lethal concentration (8 log cfu/mL), and decreasing in base 10 until reaching the minimum which caused a 100% of mortality. To study the effective dose 50 (ED50) of UB-8902, we used a model of peritoneal sepsis with groups of 10 animals with an inoculum similar to the MLD of the strains, and treated with a dose of UB-8902 from 0.5 mg/kg, increasing in base 2, to LD0 or until a 50% of survival was reached.
Results: MICs (mg/L): Ab58, CIP = 0.25, MOX=0.016, UB-8902=0.03; Ab661, CIP=8, MOX=1, UB-8902=0.5. In time-kill curves for Ab58, bactericidal activity was observed from 4 h with 4xCMI of UB-8902, from 8 h with 4xCMI of CIP, and there was no bactericidal activity with MOX. For Ab661, bactericidal activity was observed from 4 h with 4xCMI of UB-8902, from 4 h with 4xCMI and from 8 h with 2xCMI of CIP, and from 8 h with 2xCMI and 4xCMI of MOX. The toxicity parameters of UB-8902 (mg/kg) were: LD0=512, LD50=608.5, LD100=2048. The MLD was 7.5 log cfu/mL for both strains, being BLD50 of Ab58 6.11 log cfu/mL and 7 log cfu/mL for Ab661. The ED50 of UB-8902 for Ab58 was 16 mg/kg, and for Ab661 was 128 mg/kg.
Conclusions: The new quinolone UB-8902 presents bactericidal activity against *A. baumannii* resistant to ciprofloxacin and is effective in reducing the mortality in a model of murine peritoneal sepsis, with a dose lower than the toxic one, which warrants the future assessment of its efficacy in other in vivo experiments, such as a discriminative murine model of pneumonia.

P552 Intracellular penetration and activity of the new fluoroquinolone UB-8902 in human polymorphonuclear leukocytes

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Background: UB-8902, a 7-(4-methyl)-piperazine ciprofloxacin derivative, is a new fluorquinolone that shows higher in vitro activity than ciprofloxacin. The uptake of UB-8902 by human polymorphonuclear leukocytes (PMN) and its intracellular activity against isogenic strains of *Staphylococcus aureus* with sequential mutations in gyrA and/or parC genes were also evaluated.
Methods: Uptake of UB-8902 by PMNs was determined by a fluorometric assay. The effect of cell viability, environmental conditions, metabolic inhibitors, potential competitive substrates and PMN-stimuli on intracellular uptake was also studied. The intracellular activity was determined by incubation of PMN containing intracellular bacteria in the presence of UB-8902. Wild-type and mutant *S. aureus* with mutation in gyrA and/or parC were used for the killing assays.
Results: UB-8902 uptake by PMN was rapid and saturable at extracellular concentrations higher than 10 mg/L. At extracellular concentrations of 2 mg/L, the cellular to extracellular concentration (C/E) ratio was 6.5 ±0.8. The efflux of UB-8902 from PMN was also a rapid process. The intracellular penetration of UB-8902 was slightly increased when dead cells were used (7.6±1.3 versus control 6.5±0.8) and unaffected at 4ºC (C/E ratio 6.3±1.5). The uptake of UB-8902 by PMN was significatly affected by the extracellular pH (intracellular penetration decreased at basic pH and increased at acid pH). Sodium cyanide, an inhibitor of mitochondrial oxidative metabolism, significantly impaired intracellular penetration of UB-8902 (C/E ratio: 3.3±1.4). The ingestion of opsonised zymosan significantly increased the levels of PMN-associated UB-8902 (C/E ratio: 9.9± 1.3). Neither cell stimulation by phorbol myristate acetate (PMA) nor ingestion of opsonised *Staphylococcus aureus*, modified the uptake (C/E ratio:7.1±0.8 and 7.0±1.0 respectively). At extracellular concentrations evaluated, 0.125, 1 y 5 mg/L, UB-8902 significantly decreased the intracellular survival of wild-type *S. aureus*. For the mutant strains, UB-8902 only showed significant intracellular activity at extracellular concentration higher than 1 mg/L.
Conclusions: UB-8902 penetrates into PMN, reaching high intracellular concentrations and it remains active intracellularly against *S. aureus*.

P553 Definition of wildtype MIC distributions for targeted species for determination of optimal dosing for zabofloxacin, a novel fluoronaphthyridone

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Objectives: Zabofloxacin (formerly DW-224a) is a new quinolone-like agent with potent activity against pathogens responsible for

respiratory tract and uncomplicated skin and skin structure infections (*S. pneumoniae* [SPN], *H. influenzae* [HI], *M. catarrhalis* [MCAT] and *S. aureus* [SA]). Zabofloxacin was evaluated by CLSI MIC methods for subsequent PK/PD target attainment (TA) analysis to optimise doses used in various clinical trials.

Methods: Recent (2006) wildtype clinical isolates from worldwide locations (Europe, Asia, North and South America) were susceptibility (S) tested by CLSI methods: SPN (225; 200 wildtype, 25 levofloxacin-resistant [LEVO-R]); SA (200 wildtype, 200 MRSA and LEVO-R; 25 CA-MRSA); HI (55 wildtype); MCAT (10 wildtype) and CoNS (40 wildtype with 20 LEVO-R). Zabofloxacin was compared to LEVO, gemifloxacin [GEMI] and moxifloxacin [MOXI]. PK/PD studies of TA were calculated by Monte Carlo simulation from this MIC distribution for doses 50–800 mg daily (protein binding at 77%).

Results: Wildtype SPN and SA (MIC90, 0.03 mg/L); and HI and MCAT (MIC90, 0.015 mg/L were very zabofloxacin-S. In contrast, LEVO-R SPN (MIC90, 1 mg/L) and MRSA (MIC90, >8 mg/L) had higher zabofloxacin MIC values. CA-MRSA were zabofloxacin-S (also LEVO-S). Beta-lactamases and PBP-mediated resistances did not adversely influence zabofloxacin potency. Zabofloxacin (MIC50, 0.015 mg/L) was two- and eight-fold more potent than GEMI and MOXI versus wildtype penicillin-R SPN. 90% TA was achieved against SPN, HI, and MCAT wildtype pathogens (MIC, ⩽0.06 mg/L; ⩾300 mg/day) and many LEVO-R SPN.

Target pathogen (no.)[a]	Zabofloxacin MIC (mg/L)			MIC 90% (mg/L)		
	50%	90%	Range	GEMI	LEVO	MOXI
SPN, WT (200)	0.015	0.03	⩽0.0004–0.06	0.03	1	0.25
SPN, PCN-R (101)	0.015	0.03	0.008–0.06	0.03	1	0.25
SPN, LEVO-R (25)	0.12	1	0.06–8	1	>8	8
HI, WT (55)	0.008	0.015	⩽0.004–0.03	0.008	0.015	0.03
MCAT, WT (10)	0.015	0.015	0.008–0.015	0.015	0.03	0.06
MSSA, LEVO-S (200)	0.03	0.03	0.008–0.12	0.03	0.25	0.06
MSCoNS, LEVO-S (20)	0.03	0.03	0.008–0.03	0.03	0.25	0.12

a. PCN = penicillin, WT = wildtype.

Conclusions: Zabofloxacin was two- to 16- and two- to >64-fold more active than MOXI or LEVO, respectively; equal to GEMI versus RTI pathogens and wildtype MSSA. Optimal dosing appears to be achievable due to high zabofloxacin potency versus these 755 contemporary clinical strains. Clinical trial designs and PK/PD-based dosing regimens will be optimised for this promising, new orally administered agent.

P554 Tigecycline in intensive care unit infections

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Objectives: Tigecycline is a novel broad-spectrum glycylcycline antibiotic, which possesses activity against a broad range of Gram-positive, Gram-negative, atypical, anaerobic and antibiotic-resistant bacteria. It has been approved for complicated infections of skin and/or subcutaneous tissues and complicated intra-abdominal infections. For compassionate use it has been given for the treatment of infections caused by multi-resistant organisms. The aim of this study was to assess the efficacy of tigecycline in ICU infections caused by strains resistant to other antibiotics.

Methods: This is a prospective study conducted in the 18-bed medical–surgical ICU of "ATTIKON" university general hospital. Patients with severe ICU infections treated with tigecycline were included. Tigecycline was used either in cases of infections caused by pan-drug resistant Gram-negative microorganisms, including colistin, or as empirical treatment in patients with life-threatening infections which did not respond in other treatment or were colonised by PDR pathogens. Data recorded included demographic characteristics; site of infection, other antibiotics used concomitantly, treatment results after fifteen days follow up, outcome and adverse drug reactions.

Results: Between 12/ 2006 and 11/ 2007 seventeen patients were treated with tigecycline. Among them nine patients were treated for ventilator associated pneumonia (VAP), five for complicated skin or intra-abdominal infection and three for septic shock of unknown origin. Ten patients were treated empirically. Three patients received tigecycline as monotherapy and fourteen received other antimicrobials simultaneously. Seven cases of VAP were due to *Acinetobacter baumannii* alone and two cases were mixed respiratory infections (*Acinetobacter* b plus *Klebsiella* pn) and all of them were cured. Among the rest, one patient was cured and a temporal improvement was noted in 4/7 patients. Most of pathogens were susceptible only to colistin and tigecycline and four patients were infected with colistin-resistant strains. Dose was 50 mg × 2 or 100 mg × 2 IV without adverse effects.

Conclusions: Though the number of patients is small, our results are encouraging about tigecycline use for the treatment of ICU serious infections especially in ventilator-associated pneumonia. Given the problem of MDR pathogens in ICUs, tigecyclin could be an effective treatment choice. However more experience is urgently required.

P555 Anti-inflammatory effect of lycopene on chronic bacterial prostatitis rat model

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Purpose: Chronic bacterial prostatitis (CBP) is the most common urological disease in adult males, with antibiotic therapy being the gold standard for its treatment. However, long-term therapy results in many side effects as well as bacterial resistance. For these reasons, there is a need for a new treatment modality to replace traditional antibiotic therapy. Lycopene, an extract of tomatoes, has antioxidant effects against various bacteria and synergistic effects with antibiotics. We evaluate the synergistic effects of lycopene on the treatment of CBP in an animal model.

Materials and Methods: Forty five rats demonstrating CBP were randomly divided into 4 groups; the control, lycopene, ciprofloxacin and lycopene with ciprofloxacin groups. All drug treatments were conducted over a period of 2 weeks. After treatment, the results were analysed, with the microbiological cultures and histological findings of the prostate and urine samples compared with the control group and between each group.

Results: The uses of ciprofloxacin, and lycopene with ciprofloxacin showed statistically significant decreases in bacterial growth and improvements in prostatic inflammation compared with the control group ($p < 0.05$). The lycopene with ciprofloxacin group showed a statistically significant decrease in bacterial growth and improvements in prostatic inflammation compared with the ciprofloxacin group ($p < 0.05$).

Conclusions: These results suggest that lycopene may be an effective material in the treatment of CBP. Especially, the combination treatment of lycopene and ciprofloxacin has synergistic effects. Therefore, it is suggest that the combination of lycopene and ciprofloxacin may be effective in the treatment of CBP, and with a higher success rate.

P556 Efficacy of doripenem against *Pseudomonas aeruginosa* in nosocomial pneumonia, including ventilator-associated pneumonia

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Objectives: Doripenem (DOR, a new carbapenem for which an MAA has been submitted in the EU) has potent activity against bacterial pathogens commonly responsible for nosocomial pneumonia, including ventilator-associated pneumonia. In two open-label, multicentre clinical trials of patients with nosocomial pneumonia, the efficacy of DOR against *P. aeruginosa* was compared with piperacillin/tazobactam (PTZ) in non-ventilator-associated pneumonia and early-onset (<5 days) ventilator-associated pneumonia patients (Study 1), and to imipenem (IMI) in early-onset and late-onset (⩾5 days) ventilator-associated pneumonia patients (Study 2).

Methods: Adults in Study 1 were randomised to treatment for 7–14 days with 1-h infusion of IV DOR 500 mg q8h or 30-min infusion of IV PTZ 4.5 g q6h. After 72 h of IV study drug, patients were allowed to be switched to oral levofloxacin 750 mg QD. Adults in Study 2 were randomised to treatment with 7–14 days of IV DOR 500 mg q8h as a 4-h infusion or IV IMI 500 mg q6h for 30 min or 1000 mg q8h for 60 min. No oral switch was allowed. Clinical cure rates at the end of treatment were measured in the microbiologically evaluable patients (those who completed the study and had at least one bacterial lower respiratory tract pathogen identified at baseline that was susceptible to the study drug received).
Results: Overall clinical cure rates in microbiologically evaluable patients were 82.1% (69/84) with DOR versus 78.3% (65/83) with PTZ, and 69% (80/116) with DOR versus 64.5% (71/110) with IMI. The pooled clinical cure rates from both studies in microbiologically evaluable patients with *P. aeruginosa* was 81.6% (31/38) with DOR versus 54.8% (17/31) in the pooled comparators. The 95% CI of the 26.7% difference (2.4; 51.1) did not cross 0. The comparison within each trial was 83.3% [15/18] DOR versus 70.6% [12/17] PTZ (Study 1) and 80.0% [16/20] DOR versus 42.9% [6/14] IMI (Study 2).
Conclusions: DOR is a potent new carbapenem that, in a post hoc analysis of pooled data, showed significantly higher cure rates than its comparators against *P. aeruginosa* in nosocomial pneumonia, including ventilator-associated pneumonia.

P557 XF-73, a novel anti-staphylococcal antimicrobial with very rapid bactericidal activity

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Objectives: XF-73 is the lead compound in an entirely new class of anti-microbial agents, developed to address the growing and unmet need for prophylaxis and treatment of drug resistant bacteria. It has previously been shown to be highly potent against a range of *S. aureus* strains including meticillin-sensitive *Staphylococcus aureus* (MSSA), healthcare associated meticillin resistant *S. aureus* (HA-MRSA) and community associated meticillin resistant *S. aureus* (CA-MRSA) strains. XF-73 exerts rapid bactericidal activity and the aim of this study was to further characterise this effect.
Methods: Time-kill studies for XF-73 were conducted on *S. aureus* SH1000 at 4x MIC and samples were taken and prepared for scanning electron microscopy. Membrane integrity in the presence of XF-73 was determined using the BacLight assay, which evaluates the ability of propidium iodide to penetrate into the cell following 10 minutes of drug exposure. A luciferin/luciferase assay was performed to quantify XF-73-induced leakage of intracellular ATP.
Results: SH1000 cell numbers decreased by 4.5 logs following 5 minutes exposure to 4x MIC of XF-73, and by 7 logs following 30 minutes exposure. Scanning electron micrographs following 5 and 30 minutes exposure to XF-73 showed no morphological damage of SH1000 cultures, compared with untreated cells. Nevertheless, treatment for 10 minutes with XF-73 caused complete perturbation of membrane integrity as evidenced by a 100% loss of membrane integrity in the BacLite assay compared to 0% in the absence of XF-73. ATP leakage was not observed following exposure to XF-73.
Conclusion: The rapid bactericidal activity of XF-73 is not associated with significant cellular lysis. This is consistent with the absence of morphological damage to *S. aureus* cultures exposed to XF-73 and the lack of leakage of intracellular material (ATP) after a period where cell viability is reduced by 7 logs. Membrane integrity was however perturbed, suggesting that although the cell remained physically intact, the membrane was severely damaged. Investigations are ongoing to elucidate further the mechanism of action of this highly potent and rapidly bactericidal anti-bacterial agent.

P558 XF-73: a novel antimicrobial – Investigation of the change in MIC against multiple MRSA strains during a 55-passage study

W. Rhys-Williams, W. Love, I. Hayter, D. Farrell, R. Janes, M. Robbins (Brighton, London, UK)

Objectives: XF-73, the lead compound in a new class of anti-microbial agents, has previously been shown to be potent (MIC50 1 mg/L) against a range of *Staphylococcus aureus* strains including meticillin-sensitive *S. aureus* (MSSA), healthcare-associated meticillin-resistant *S. aureus* (HA-MRSA) and community-associated meticillin-resistant *S. aureus* (CA-MRSA). XF-73 is bactericidal (MBC50 1 mg/L) and the aim of this study was to investigate the propensity of XF-73 to cause mutational resistance in common MRSA clones including USA300, responsible for the majority of CA-MRSA infections in the USA. An antibiotic comparator (fusidic acid) was also studied.
Methods: MICs were determined for XF-73 and fusidic acid using a macrodilution broth method. Five "Network on Antimicrobial Resistance in *Staphylococcus aureus*" (NARSA) strains were tested: NRS382, NRS384 (USA 300), NRS271, NRS123, NRS387. All 5 strains were passaged 55X at 0.5X MIC (determined from the previous passage) to investigate whether there was an increase in the MIC, suggestive of the development of mutational resistance.
Results: The initial MICs of XF-73 and fusidic acid were found to be 0.25–0.5 mg/L and 0.12–0.25 mg/L respectively against the 5 MRSA strains tested. For all 5 strains, a rapid increase in the MIC of fusidic acid was observed after only 2–5 repeat passages, with the MIC reaching 256 mg/L after 33–42 passages. In comparison, the MIC of XF-73 did not increase significantly (<8-fold increase), even after 55 passages.

	XF-73 MIC (mg/L)					Fusidic acid MIC (mg/L)				
	NRS 123	NRS 271	NRS 382	NRS 384	NRS 387	NRS 123	NRS 271	NRS 382	NRS 384	NRS 387
Passage 0	0.5	0.25	0.25	0.25	0.25	0.12	0.25	0.12	0.12	0.12
Passage 55	1	0.5	1	0.5	0.5	256	256	256	256	256

Conclusion: Mutational resistance to fusidic acid in *S. aureus* is an important resistance mechanism. In the UK and Australia, susceptibility to fusidic acid is defined as an MIC of ⩽0.25 mg/L or ⩽0.5 mg/L respectively and resistance is defined as an MIC of 2 mg/L or more. All 5 MRSA strains rapidly developed resistance to fusidic acid after only a few repeat passage exposures. In comparison, with XF-73 there was no significant increase in MIC against all five selected MRSA strains, even after 55 repeat passages. This suggests that the likelihood for mutational resistance development against XF-73 is remote and hence demonstrates potential for a long clinical lifetime.

P559 XF-73: a novel antimicrobial with broad-ranging Gram-positive antibacterial activity

W. Love, W. Rhys-Williams, I. Hayter, D. Farrell, J. Curry, M. Robbins (Brighton, London, UK)

Objectives: XF-73, the lead compound in a new class of anti-microbial agents, has previously been shown to be potent (MIC50 1 mg/L) against a range of *Staphylococcus aureus* strains including meticillin-sensitive *S. aureus* (MSSA), healthcare-associated meticillin resistant *S. aureus* (HA-MRSA) and community-associated meticillin resistant *S. aureus* (CA-MRSA). XF-73 is bactericidal (MBC50 1 mg/L) and the aim of this study was to investigate its anti-bacterial activity against a range of aerobic and anaerobic Gram-positive bacteria.
Methods: MICs for XF-73 were determined against a panel of 65 Gram-positive bacterial strains (56 aerobic and 9 anaerobic) using the CLSI

broth microdilution methodology. XF-73 was used in a log2 dilution series from 0.03 to 64 mg/L. Where possible, both antibiotic resistant and susceptible strains were included.
Results: XF-73 was found to have antibacterial activity (MIC range 0.25–4 mg/L) against all 65 strains tested. The results are provided in the table.

Species (No. of isolates)	Characteristic/Phenotype/Genotype	XF-73 MIC Range (mg/L)
Staphylococcus aureus (15)	MSSA, MRSA, VISA	0.5–1
Coagulase-negative *Staphylococcus* spp. (5)	MSSE, MRSE, *S. haemolyticus*, *S. saprophyticus*	0.25–1
Enterococcus spp. (9)	Vancomycin susceptible, VanA, VanB, VanC	0.5–2
Beta-haemolytic *Streptococcus* spp. (9)	Macrolide susceptible and resistant clinical isolates	0.25–2
Alpha-haemolytic *Streptococcus* spp. (15)	Penicillin and/or macrolide resistant, multi-resistant clinical isolates	0.5–4
Corynebacterium jeikeium (2)	Clinical isolates	Both 0.25
Listeria monocytogenes (1)	Clinical isolate	0.5
Gram-positive anaerobic species (9)	*Clostridium*, *Peptostreptococcus* and *Propionibacterium* spp.	0.5–4

Conclusion: XF-73 demonstrated potent activity against all of the aerobic and anaerobic Gram-positive bacteria tested. These initial results suggest that XF-73 has potential for use as a novel antibacterial agent against a wide range of clinically relevant Gram-positive pathogens. Studies are ongoing to investigate the use of XF-73 in a range of different bacterial disease indications.

P560 In vitro and in vivo activity of SB006, a novel branched antimicrobial peptide selectively active against Gram-negative bacteria.

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Objectives: The aim of this study was to investigate the in vitro and in vivo activity of SB006 against selected MDR Gram-negative clinical isolates.
Methods: MICs and MBCs were determined in MHB by the broth microdilution methodology, according to CLSI procedure. MIC was defined as the lowest drug concentration causing complete suppression of visible bacterial or fungal growth. MBC was defined as the antibiotic concentration that induced at least a 3 log reduction of the initial bacterial inoculum.
The in vivo activity of SB006 has been evaluated using a neutropenic mouse model of infection. Mice were infected intraperitonally using a 0.2-ml volume of a high dose inoculum (3xLD50) of *Pseudomonas aeruginosa* (clinical isolate). Several single dosages of SB006 were administered at 1 hour post-challenge. The total dose required for survival of 50% of mice at 72 h (ED50) was determined.
Results: The in vitro activity of SB006 and reference compounds against an expanded panel of clinical bacteria recently collected has been determined; all tested strains show resistance to several currently used antibacterial agents. SB006 was active against Gram-negative bacteria; the MICs of SB006 against *Acinetobacter* spp. (2 strains), *Enterobacter* spp. (4 strains), *Escherichia coli* (12 strains), *Klebsiella pneumoniae* (6 strains), *Pseudomonas aeruginosa* (16 strains), *Salmonella enteritidis* (1 strain), and *Stenotrophomonas maltophilia* (1 strain) were between 4 and 32 mg/L, while colistin showed MICs ranging between 0.25 and 8 mg/L. The MIC and MBC values of SB006 ranged between 8 and 32 mg/L, while those of colistin ranged between 0.25 and 4 mg/L. The MBC/MIC ratio was always 1 for SB006 and colistin. Moreover, in vivo studies showed that SB006 was active in an experimental mouse model of infection against a clinical isolate of *P. aeruginosa*.
Conclusion: SB006 showed good activity against different Gram-negative bacteria isolates with a potency and spectrum of antimicrobial activity comparable to those of colistin and showed to be bactericidal. As colistin, SB006 seems to selectively perturb the bacterial membrane: this mechanism of action is expected to circumvent the problem of resistance. These features make the antimicrobial branched peptide SB006 a promising template for the development of new antibacterial drugs.

P561 SASP: rapid bactericidal activity against USA strains of meticillin-resistant *Staphylococcus aureus*

A. Barnard, K. Pitts, D. Brown, A. Wilkinson, H. Fairhead (Cambridge, UK)

Objectives: SASP is a unique antibacterial protein that acts by binding to bacterial DNA and halting replication and gene expression, resulting in rapid cell death. SASPjectTM is a novel platform technology by which a SASP gene is delivered to selected bacteria via dedicated bacteriophage vectors. Previous studies using *S. aureus*-specific SASPjectTM vector, PTSA1.2/A, showed SASP is rapidly bactericidal (assessing kill at 3 h) against 163 geographically diverse clinical MRSA isolates spanning SCCmec types 1-V (10^5 cfu/ml). In this study, efficacy of SASP against MRSA strains isolated in the USA is determined by time kill assay over 24 h at higher cell densities (10^7 cfu/ml). Speed of kill of SASP against an MRSA USA300 isolate is also assessed.
Methods: Ten different MRSA isolates of different USA types were tested for susceptibility to SASP by time kill assay. Bacterial cells were grown overnight in Luria-Bertani broth supplemented with calcium chloride (10 mM), diluted to 2 x 10^7 cfu/ml and mixed with an equal volume (500 μl) of PTSA1.2/A (6 x 10^7 pfu/ml). Cultures were incubated at 35ºC, samples were removed at 0, 1, 2, 4, 6 and 24 hours and viable cells were enumerated following overnight incubation on blood agar.
Speed of kill was assessed by the same method against a clinical MRSA USA300 isolate diluted to 5 x 10^5 or 5 x 10^7 cfu/ml with viable counts performed at 0, 2, 5, 10, 15, 20, 30, 45 and 60 mins. For all controls, PTSA1.2/A was replaced with buffer.
Results: SASP was rapidly bactericidal against all 10 clinical isolates tested, with 5 isolates showing a >3-log drop in viability within 1 h and remaining isolates showing a >3-log drop in viability within 2 h.
In the speed of kill assay, SASP caused a >99.9% drop in viability within 5 mins against the 10^5 culture and a >99.9% drop in viability within 10 mins against the 10^7 culture.
Conclusions:
- SASPject vector, PTSA1.2/A can effectively deliver a SASP gene to all tested MRSA strains.
- SASP shows rapid bactericidal activity against tested MRSA strains covering different USA types.

P562 The efficacy of SASP targeted to meticillin-resistant *Staphylococcus aureus* in mixed staphylococcal cultures

K. Pitts, A. Barnard, D. Brown, A. Wilkinson, H. Fairhead (Cambridge, UK)

Objectives: SASPjectTM is a novel platform technology which allows bacterial species to be targeted with the SASP anti-bacterial protein by means of a species-specific delivery vector, without affecting the body's normal microbial flora. This study utilised a *Staphylococcus aureus*-specific SASPjectTM delivery vector, PTSA1.2/A, developed for intra-nasal elimination of MRSA. SASP has previously demonstrated rapid bactericidal activity against a wide range of clinical MRSA and meticillin susceptible *S. aureus* (MSSA) isolates. In this study the effect of co-cultures of MRSA, MSSA, *S. epidermidis* and *S. haemolyticus* on the efficacy of SASP is assessed.
Methods: *S. epidermidis* was grown overnight in Luria-Bertani broth and mixed (10^7 cfu/ml final concentration) with a clinical MRSA strain at final concentrations of 10^4, 10^5, or 10^6 cfu/ml. PTSA1.2/A (6 x 10^8 pfu/ml) was then added. Mixed cultures containing MRSA and a clinical MSSA or *S. haemolyticus* strain were similarly prepared and again supplemented with PTSA1.2/A. Appropriate mono-cultures and

PTSA1.2/A controls were also assayed. Cell viability was measured over time by performing cell counts at 0, 1, 2, 4 and 6 hours.
Results: The presence of *S. epidermidis* did not affect the rate of kill of MRSA by PTSA1.2/A, with no detectable survivors by 2 h for any of the 3 MRSA cell concentrations tested±*S. epidermidis*. Furthermore, *S. epidermidis* grew at the same rate in the presence or absence of PTSA1.2/A. A similar trend was seen with *S. haemolyticus*. PTSA1.2/A killed both MRSA and MSSA cells when co-cultured (no detectable survivors by 4 hours) and the rate of kill of MRSA (>3 log drop in viability in 2 h) was comparable in the presence or absence of MSSA.
Conclusions:
- SASP delivered by PTSA1.2/A is rapidly bactericidal against MRSA.
- Rate of kill, in the combinations tested, is unaffected by the presence of other staphylococcal species commonly found in the human nose.
- The presence of PTSA1.2/A does not affect the growth rate of other staphylococcal species, indicating that the SASPjectTM system can be used to target selected bacteria whilst leaving the normal flora intact.

P563 Evaluation of the type I signal peptidase of *Staphylococcus epidermidis* as novel antibacterial target

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Objectives: In a time of emerging bacterial resistance there is need for novel antibacterial targets. One of the bacteria of concern is *Staphylococcus epidermidis*, an opportunistic pathogen associated with foreign body infections and nosocomial sepsis.
We aim to develop antimicrobial drugs against a new target, the type I signal peptidase (SPase I). SPases I have a role in protein secretion as they remove the signal peptides from secretory proteins during or after translocation across the membrane and have been shown to be essential for the viability of the cell. *S. epidermidis* has three type I signal peptidases, Sip1, Sip2 and Sip3.
We therefore aim to evaluate the SPase I of *S. epidermidis* as a novel antibacterial target and to design, synthesize and evaluate the inhibitory activity of potential SPase I inhibitors.
Methods: For all DNA manipulations standard techniques were used. SDS-PAGE was used to analyse the overproduction of the enzymes in *Escherichia coli* after IPTG induction. Purification of His tagged enzymes was performed under native conditions using affinity chromatography on a Ni2+-NTA agarose column. SPase expression in planktonic and sessile grown *S. epidermidis* was analysed by RT-PCR experiments.
Substrate analogues were rationally designed (by modelling using the structure for the *E. coli* signal peptidase) and synthesised by solid phase peptide synthesis using classical coupling reagents for peptide bond formation and Fmoc protected amino acids. The synthesised compounds were purified by Reversed Phase-High Performance Liquid Chromatography and analysed by High Resolution Mass Spectrometry.
Inhibitory activity against SPase I was evaluated by an in vitro inhibitor assay based on intramolecular fluorescent resonance energy transfer (FRET) using an internally quenched fluorescent peptide substrate based on a *S. epidermidis* preprotein.
Results: The SPase I genes of *S. epidermidis* were cloned, expressed and purified in *E. coli* and biochemically analysed. The enzymes were used to develop and optimise a FRET based in vitro assay, allowing evaluation of potential SPase I inhibitors such as rationally designed peptide substrate analogues.
Conclusion: Due to their unique properties, SPases I seem to be an attractive target for the development of new antimicrobial drugs. A FRET based in vitro assay was developed and used for the evaluation of potential SPase I inhibitors.

P564 Disruption of *Pseudomonas aeruginosa* quorum sensing using high-affinity antibodies derived from immunised sheep

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Objectives: *Pseudomonas aeruginosa* is an opportunistic human pathogen which, like many other Gram-negative pathogens, employs quorum sensing – regulated virulence factors to establish an infection in its host. The bacterium is notorious for antibiotic resistance and causes life-threatening infections in immunocompromised and cystic fibrosis patients, and also accounts for 11% of nosocomial infections worldwide. Quorum sensing in *Ps. aeruginosa* populations is controlled by low molecular weight (hapten) signalling molecules known as homoserine lactones (HSLs). Previous work in our lab has demonstrated that sheep are capable of producing high affinity, high specificity anti-hapten antibodies, so we sought to develop anti-HSL antibodies derived from immunised sheep and explore their potential as novel antibacterial agents.
Methods: Derivatives of *Ps. aeruginosa* HSL molecules were synthesised, conjugated to suitable carrier proteins, and used to elicit an immune response in sheep. Post immunisation, sheep polyclonal sera were analysed using immunoassays to determine the level of specific immune response. Bioassays were also employed to demonstrate the abilities of the sera to block *Ps. aeruginosa* quorum sensing.
Results: The ability of immunised sheep polyclonal serum to bind specifically to quorum sensing molecules has been demonstrated by a series of in vitro immunoassays and bioassays. Immunisation with a mixture of three HSL subgroups resulted in high antibody titre polyclonal serum which showed nanomolar affinities to the free form of HSLs when applied in a competition format.
Conclusions: Antibodies are an attractive method for controlling bacterial virulence and biofilm formation in Gram-negative bacteria, and at the same time these ‘antipathogenic’ drugs are less likely to develop resistance in bacteria compared to conventional antibiotics.
The results from preliminary experiments are highly encouraging and demonstrate the possibility of isolating nanomolar affinity anti-HSL antibodies from immunised sheep. Phage display antibody libraries are currently being constructed from sheep peripheral blood lymphocytes, and will be screened in due course for anti-HSL monoclonal antibodies.

P565 Interaction of PZ-601 (SMP-601) and other β-lactams with chromosome- and plasmid-encoded class C β-lactamases

J.D. Docquier, L. Xerri (Siena, IT; Malvern, US)

Objectives: The overproduction of chromosome-encoded class C β-lactamases in derepressed mutants of Enterobacteriaceae and *Pseudomonas aeruginosa* is a known mechanism able to compromise the efficacy of penicillins and cephalosporins for the treatment of infections caused by these organisms. More recently, the emergence of plasmid-encoded class C β-lactamases, currently spreading in Enterobacteriaceae, is of increasing concern. We carried out a comparative study on the interaction of PZ-601 (a novel carbapenem with a spectrum including multidrug resistant Gram-positive pathogens) with several class C enzymes.
Methods: The stability of PZ-601, imipenem and cephalosporins to hydrolysis by P99 AmpC, CMY-2 and FOX-7 β-lactamases was investigated spectrophotometrically, using either direct hydrolysis measurements or the reporter substrate method. Antimicrobial susceptibilities of clinical isolates and transconjugants (or transformants) thereof were measured using the microdilution broth method as recommended by the CLSI.
Results: In enzyme assays, PZ-601 behaved as a poor substrate of these enzymes and, similarly to imipenem (IPM), showed degradation rates slower than those observed with tested oxyimino-cephalosporins cefotaxime (CTX) and ceftazidime (CAZ), and piperacillin (PIP), as reflected by the higher catalytic efficiencies measured with the latter compounds. These data were confirmed by the changes in the antimicrobial susceptibility profiles when a CMY-2-encoding plasmid was transferred into an *E. coli* laboratory strain (J53). This transformed

strain gained resistance to CAZ, CTX and PIP, while it showed only a reduced susceptibility to PZ-601.

Strain	β-lactamase	MICs (mg/L)				
		PZ-601	IPM	CAZ	CTX	PIP
E. coli J53	None	0.25	0.12	0.12	0.06	1
E. coli J53 (p26Sm02)	CMY-2	2	0.12	64	16	>512
E. coli 26Sm02	CMY-2	4	0.25	64	32	>512
Enterobacter cloacae RM-SI	AmpC	8	0.5	64	64	32
Klebsiella pneumoniae Kpn7	FOX-7	8	0.25	256	32	>512

The parental clinical isolates all exhibit the lower MIC values with IPM and PZ-601, while a high level of resistance was observed with the other agents. It is likely that for PZ-601, permeability defects in these isolates may act synergistically with a slow β-lactamase degradation, explaining in part the different susceptibility to the latter, as compared to IPM.
Conclusion: PZ-601 was confirmed to have a better stability to class C β-lactamases than tested oxyimino-cephalosporins and penicillins, even though it appeared to be slightly less stable to these enzymes than imipenem.

P566 Stability of PZ-601 (SMP-601) to different clinically relevant extended-spectrum β-lactamases

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Objectives: PZ-601 is a new carbapenem showing activity against meticillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci. PZ-601 has been shown to exhibit good activity versus Gram-negative pathogens producing extended-spectrum β-lactamases (ESBLs) (Paterson et al.; Lolans et al., ICAAC 2007). The stability of PZ-601 to various clinically-relevant enzymes is investigated and compared to that of other β-lactams.
Methods: The stability of PZ-601 to the hydrolytic action of representative TEM-, SHV-, CTX-M- and GES-types ESBLs was investigated spectrophotometrically, using either direct hydrolysis measurements or the reporter substrate method. In the latter case, inactivation parameters were computed from enzyme inactivation time courses in the presence of various carbapenem concentrations. Susceptibilities to PZ-601, imipenem (IPM), ceftazidime (CAZ), cefepime (FEP) and piperacillin (PIP) were measured (microdilution broth method, CLSI) for recombinant strains carrying the cloned β-lactamase gene and clinical isolates with known β-lactamase content.
Results: In enzyme assays, PZ-601 behaved as a transient inactivator of all tested β-lactamases. It showed an excellent stability to these enzymes, as reflected by extremely low deacylation rates. Interestingly, PZ-601 was more stable than IPM to GES-1, behaving as a poor substrate of this enzyme. MIC values of PZ-601 were not affected by β-lactamase production in laboratory strains of *E. coli* expressing the cloned β-lactamase gene, in comparison with the control strain carrying the empty cloning vector.

Strain	ESBL	MICs (mg/L)				
		PZ-601	IPM	CAZ	FEP	PIP
E. coli DH5α(pBC-SK)	None	0.25	0.12	0.12	0.06	1
E. coli DH5α(pLBII-TEM-72)	TEM-72	0.25	0.12	64	4	512
E. coli DH5α(pLBII-SHV-12)	SHV-12	0.25	0.25	256	8	256
E. coli DH5α(pLBII-CTX-M-1)	CTX-M-1	0.25	0.25	1	4	64
E. coli DH5α(pBC-GES-1)	GES-1	0.25	0.5	2	0.12	64

The determination of the antimicrobial susceptibility profiles of the clinical isolates producing the same ESBLs revealed an in vitro efficacy of PZ-601 similar to that of IPM, while higher MIC values were expectedly recorded for third- and fourth-generation cephalosporins and penicillins.
Conclusion: Due to its stability to most types of clinically-relevant ESBLs, PZ-601 is active on both ESBL-producing laboratory strains and their parental clinical isolates. PZ-601 might represent a valuable alternative drug for empiric therapy when broad-spectrum antimicrobial activity, including both multidrug-resistant Gram-positives and Gram-negatives, is required.

P567 New thioureides of 2-(4-methyl-phenoxymethyl) benzoic acid with antimicrobial activity

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Objectives: The present application is a continuation of our research concerning the synthesis and characterisation of 2-(4-methyl-phenoxymethyl)-benzoic acid thioureides with antimicrobial activities.
The aim of this study was to evaluate the in vitro antimicrobial activity of some newly synthesised chemical compounds using qualitative and quantitative methods.
Methods: The new thioureides were obtained in three stages: first, the 2-(4-methyl-phenoxymethyl)benzoic acid by reacting sodium p-methyl-phenoxide with phtalide, then the synthesis of the 2-(4-methyl-phenoxymethyl)benzoic acid chloride, and finally the corresponding isothiocyanate is treated with primary aromatic amines to obtain the new compounds.
The qualitative screening of the susceptibility spectra of different microbial strains to these compounds was performed by three adaptated diffusion methods: paper filter disk impregnation with the tested substances solutions, the disposal of tested solutions in agar wells and the spotting of tested solutions on solid medium seeded with microbial inoculums. The quantitative assay of the antimicrobial activity was performed by broth microdilution method in 96-well microplates in order to establish the minimal inhibitory concentration (MIC).The antimicrobial activity was tested against Gram-positive strains (*Staphylococcus* (*S.*) *aureus*, *Bacillus* (*B.*) *subtilis*), Gram-negative (*Escherichia* (*E.*) *coli*, *Pseudomonas* (*P.*) *aeruginosa*, *Klebsiella* (*K.*) *pneumoniae*) and fungal strains (*Candida* (*C.*) *albicans*, *Aspergillus* (*A.*) *niger*). All tests were performed comparatively with reference strains: *K. pneumoniae* IC 13420, *E. coli* IC 13529, *S. aureus* IC 13204, *P. aeruginosa* IC 13202, *B. subtilis* IC 12488, *C. albicans* IC 249, *A. niger* IC 13534.
Results: Chemical structures of the synthesised compounds have been elucidated by theirs 1H-NMR, 13C-NMR, IR spectra and elemental analysis.
Our results showed that the tested compounds exhibited specific antimicrobial activity, the highest activity being noticed against suspended fungal cells (MIC ranging from 62.5 to 15.6), followed by *P. aeruginosa* (MICs from 250 to 31.5). Antimicrobial activity against Gram-positive bacteria were noticed for most compounds exhibiting very low MICs (MICs from 1000 to 62.5).
Conclusion: The 2-(4-methyl-phenoxymethyl)-benzoic acid thioureides exhibited specific antimicrobial activity and may be regarded as potential antimicrobial agents.

P568 A synthetic derivative of the host immune defence peptide, bactenecin, activity against clinical isolates of meticillin-susceptible and meticillin-resistant *Staphylococcus aureus*, a potential new therapeutic agent

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Vancomycin is one of the few antibiotics still effective in the treatment of meticillin-resistant *Staphylococcus aureus* (MRSA) and treatment failures are increasingly reported with this antibiotic. Therefore, the development of alternative antibiotics with novel modes of action is critical. Cationic antimicrobial peptides with structures resembling naturally occurring host defence peptides are promising candidates. Some of the benefits of host defence peptides as antimicrobial agents

include, reduced capacity to develop resistance, multiple and novel modes of action and reduced toxicity.
Objectives: In this study, the potential of an 8 amino-acid linear peptide derived from bactenecin, an immune peptide found in bovine neutrophils, as a therapeutic agent for the treatment of MRSA, was investigated.
Methods: The minimum inhibitory concentrations (MIC) of D and L-stereoisomers of the bactenecin derived peptide, for thirteen MRSA and twelve meticillin-susceptible *S. aureus* (MSSA) isolates from bloodstream infections were determined. The effect of pH, salt concentration and cations, calcium and magnesium, on the in-vitro activity of the peptide was also investigated. The capacity for development of in-vitro resistance to the bactenecin derived peptide was also studied by sequential exposure of two MRSA isolates to the L-peptide, and comparison of the MIC of surviving colonies to the parental colonies.
Results: All isolates were susceptible to the bactenecin-derived peptide with MIC $\leqslant$ 50 μg/ml for the L-peptide and MIC $\leqslant$ 5 μg/ml for the D-peptide. Optimal bactericidal activity was reached over the pH range 7.0 to 9.0 and potassium phosphate concentration of 1–10 mM. The MIC was not affected by calcium and magnesium. Sequential exposure of two clinical isolates, seven times to a sub-inhibitory concentration of the L-peptide, did not change the MIC, suggesting that further exposure would be required to generate resistance.
Conclusions: Our study concludes that this bactenecin-derived peptide may have potential for development as an alternative anti-staphylococcal agent, as it is effective against invasive isolates of MSSA and MRSA, is active under physiological conditions and does not easily select resistant mutants.

P569 In vitro activity of a cecropin A-melittin antimicrobial peptide hybrid vs. multidrug-resistant strains of *Acinetobacter baumannii* prevalent in the UK

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Objectives: *Acinetobacter baumannii* has emerged as an important nosocomial pathogen causing burn wound infections, ventilator associated pneumonia, bacteraemia and sepsis in the critically ill. The organism exhibits formidable multi-drug resistance with the commonest isolates in the UK (OXA-23 clone 1) exhibiting resistance to all β-lactams (including carbapenems), β-lactam / inhibitor combinations, quinolones, and aminoglycosides. In view of the lack of agents in development for the treatment of MDR *Acinetobacter*s we set out to determine the in-vitro activity of a hybrid of two antimicrobial peptides, cecropin A and melittin (CAMEL) versus a collection of recent clinical isolates and determine the killing kinetics versus the OXA-23 (1) strain.
Methods: A 15 residue peptide based on residues 1–7 of cecropin A and residues 2–9 of melittin was synthesised using fmoc chemistryand purified by solid phase extraction. Sixty-six consecutive clinical isolates, identified as *A. baumannii* by API 20NE and confirmed by specific PCR for the OXA-51 gene were obtained. Inhibitory and bactericidal concentrations of CAMEL peptide were determined by microtitre dilution using according to standard BSAC methodology. Time-kill assays using Camel peptide at 1 X and 2 X the MIC were performed on a representative isolate of OXA-23 clone 1.
Results: The MIC50 of Camel peptide was 8 mg/L and the MIC90 16 mg/L with a range of 4–32 mg/L. The MBC50 and MBC90 were 16 and 32 mg/L respectively (range 8–64 mg/L). In time kill assays Camel was rapidly bactericidal within 2 hrs. At 2 × MIC >3-fold log reductions in CFU/ml were sustained over 24 hrs.
Conclusions: CAMEL peptide exhibited promising activity versus prevalent strains of multidrug resistant *A. baumannii* and was rapidly bactericidal versus the OXA-23 clone 1 of *A. baumannii*. Further studies to determine in vivo stability, bioavailability and toxicity towards eukaryotic cells are needed to enable further development of this novel compound.

P570 Resistance studies on the siderophore monobactam/β-lactamase inhibitor combination BAL30376

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Objective: Multi-resistant clinical isolates of Gram-negative bacteria were examined for their propensity to develop resistance to BAL30376, novel combination between the monobactam antibiotic BAL0019764 and the specific β-lactamase inhibitors BAL0029880 and clavulanic acid.
Methods: Isolates of *Pseudomonas aeruginosa*, *Acinetobacter* sp., *Stenotrophomonas maltophilia*, *Enterobacter cloacae*, and *Klebsiella pneumoniae* were examined for their propensity to spontaneously acquire resistance to BAL30376 and selected comparators, either in single-step selection or by repeated passaging in cultures with increasing concentrations of BAL30376.
Results: Population analysis indicated single-step resistance frequencies of 10^{-8} to 10^{-10} for BAL30376 against the strains Enterobacteriaceae and *Acinetobacter*. The frequency of appearance of spontaneous resistance to cefepime or meropenem in susceptible strains was between 10^{-6} and 10^{-9}. Subpopulations with elevated BAL30376 MICs (up to 8 mg/L) were identified in all *P. aeruginosa* strains examined, with incidence between 10^{-7} and 10^{-9}. Subpopulations resistant to cefepime or meropenem were also detected at an incidence between 10^{-5} and 10^{-7}. Repeated passaging in cultures containing BAL30376 yielded strains with >4-fold elevated MIC after 10–20 passages. Strains with >4-fold elevated MIC towards cefepime or meropenem appeared within 4 passages under the same conditions. Many of the resistant isolates obtained in these studies had increased levels of expression of β-lactamase.
Conclusion: Low frequencies of spontaneous resistance to BAL30376 were observed. Despite its complexity, the triple combination is relatively refractory to development of resistance.

P571 Bactericidal effect of the siderophore monobactam/β-lactamase inhibitor combination BAL30376

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Objective: BAL30376 is a novel combination between the monobactam antibiotic BAL0019764 and the specific β-lactamase inhibitors BAL0029880 and clavulanic acid. We have assessed the in vitro time-kill kinetics of this combination using a selection of multi-resistant Gram-negative bacteria.
Methods: MICs were determined by standard broth micro-dilution methods. A preliminary MBC was determined by plating out the clear wells from the MIC determination and counting colonies 24 and 48h after plating. Time-kill studies were done in standard growth medium with an inoculum of approximately 10^6 cfu/ml. Serial dilutions were taken a 0, 3, 6, 24 and 28 h after inoculation.
Results: MBC was 1–4 × MIC for the action of BAL30376 against most strains of Enterobacteriaceae and *Acinetobacter* sp. that were investigated. MBC could be up to 16 × MIC for the action of BAL30376 against some strains of *Pseudomonas aeruginosa*. Against the same strains, the MBCs of cefepime and meropenem were 1–4 × MIC for susceptible strains of Enterobacteriaceae and 4–>32 × MIC for susceptible strains of *Acinetobacter* and *P. aeruginosa*. Time-kill analysis demonstrated that BAL30376 decreased the cfu count by 3 orders of magnitude within 24 h at 4 and 8 × MIC against Enterobacteriaceae, *Acinetobacter* and *P. aeruginosa*. The kill kinetics were dependent on inoculum density and were faster with an inoculum of 5×10^4 cfu/ml and slower with an inoculum of 10^7 cfu/ml.
Conclusion: BAL30376 has marked bactericidal activity against a range of multi-resistant Gram-negative bacteria, including carbapenem-resistant strains.

P572 In vivo effectiveness and dose response of BAL30376 in murine peritonitis sepsis model caused by *Pseudomonas aeruginosa* or by extended-spectrum β-lactamase producing *Escherichia coli*

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Pseudomonas aeruginosa (PA) and extended spectrum beta lactamase (ESBL) *E. coli* are common nosocomial pathogens often affecting immunocompromised patients that are difficult to treat. Against these pathogens we aimed to study in a lethal mice peritonitis sepsis model of infection the in vivo efficacy of a new monobactam beta lactamase inhibitor combination, BAL30376 specifically designed to overcome most resistance mechanisms in non-fermenters and Enterobacteriaceae, including metallo betalactamases and ESBLs.

Methods: PA and ESBL *E. coli* with MIC <0.0625 mg/L for BAL30376 were used as infecting bacteria in the in vivo model. Neutropenia was induced in ICR mice (23–25 g) and peritonitis was induced by intraperitoneal inoculation of either PA or ESBL *E. coli*. Efficacy was studied at various bacterial inocula: 5×10^2 -1×10^4 cfu /mouse (1–50 LD50) for PA, and $4–8\times10^6$ cfu /mouse (4–8 LD50) for ESBL *E. coli*. Mice (n = 12) were treated with BAL30376 three times a day given ip for three days. Dose effect was studied at a range of doses between 6.25 to 100 mg/Kg. Survival was monitored for seven days post infection and compared with control groups (A saline placebo group and a meropenem treated group) using log rank test.

Results: In two sets of experiments PA peritonitis (at 6x LD50) resulted in 70–100% mortality in placebo group. BAL30376 had 100% protection at doses $\geqslant$ 25 mg/Kg ($p<0.01$) similarly to the meropenem treated group. Lower doses of BAL30376 were moderately protective. ESBL *E. coli* peritonitis was studied in two experiments (4–8x LD50) and resulted in 100% mortality in placebo group. BAL30376 had 100% protection at doses $\geqslant$ 12.5 mg/Kg ($p<0.01$). With both pathogen studied at various inocula and dosages of BAL30376 dose effect were confirmed.

Conclusions: BAL30376 was highly effective in treating lethal peritonitis and sepsis caused by PA or ESBL *E. coli* in murine model.

P573 Comparative efficacy of EDP-420, azithromycin, telithromycin, clindamycin, trimethoprim/sulfamethoxazole, linezolid and levofloxacin against murine skin abscess induced by meticillin-resistant *Staphylococcus aureus* USA300 PVL+ mecA strain

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Objectives: USA 300, a pulsed-field type of meticillin-resistant *Staphylococcus aureus* (MRSA) isolates, has demonstrated in outbreak and non-outbreak community-acquired (CA-MRSA) as well as hospital-associated *S. aureus* skin and soft-tissue infections (SSTI) which is becoming one of the biggest infectious mysteries of the decade. Investigators are not sure where it came from, where it lives, how it spreads and how to treat empirically. USA 300 had an antimicrobial susceptibility profile demonstrating resistance only to β-lactams and erythromycin while retaining susceptibility to clindamycin, levofloxacin, trimethoprim–sulfamethoxazole, and vancomycin. Guidelines for the empirical treatment of CA-MRSA on SSTI are ambiguous. EDP-420, a new bicyclolide (bridged bicyclic macrolide) with unique structural features, was sensitive against USA300 in vitro. It is necessary to compare its in vivo efficacy with major antibiotics in murine skin abscess model induced by USA300 and to determine its skin and abscess concentration (a tissue PK).

Methods: Skin abscess was induced in mice by subcutaneous inoculation of 10^7 CFU of USA300. Mice developed abscess within 1 day. Abscess bacterial counts increased progressively. Bacterial counts can reach 10^9 CFU/g skin abscess tissue if leave untreated. Treatment was initiated immediately, 24 h and 48 h after inoculation. Bacterial burden in abscess and lesion sizes were compared by the end of the experiments. Single dose pharmacokinetics of EDP-420 in plasma, normal skin and abscess tissue were compared in abscess infected mice.

Results: EDP-420 was as effective as linezolid and clindamycin in the MRSA (*S. aureus* USA300) model of murine abscess with a PD50s of 5.3, 7.3 and 10.7 mg/kg respectively. Levofloxacin and telithromycin were less active than EDP-420 with a PD50s of 20.1 and 25.9 mg/kg respectively. Azithromycin and trimethoprim/sulfamethoxazole performed poorly with a PD50s of >100 mg/kg. EDP-420 exhibited an 8 fold abscess/plasma AUC ratio. Abscess in untreated animals appeared 24 h after inoculation, and persisted for up to 2 weeks.

Conclusion: EDP-420 demonstrated better efficacy in the CA-MRSA murine abscess model in comparison with currently marketed oxazolidinone, lincosamide, fluoroquinolone, ketolide, macrolide as well as dihydrofolate reductase inhibitor/sulfonamide class antibiotics. The results indicate that EDP-420 is potentially a potent antibiotic for treatment of CA-MRSA in SSTI.

P574 Comparative effects of faropenem-medoxomil and amoxicillin-clavulanate on the normal oral and intestinal micro flora and the selection of penem-resistance

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Objectives: Community-acquired enterobacteriaceae have acquired ESBL- or AmpC-producing genes thus requiring more active β-lactams such as faropenem. However suppression of the normal flora in the oral cavity and gastro-intestinal tract may select for colonisation with resistant bacteria. Therefore we evaluated the effects of orally administered faropenem-medoxomil (FAR) or amoxicillin-clavulanate (A/C) on the normal human flora and on colonisation of the oral cavity and colon. Emergence of penem-resistance was monitored.

Methods: Healthy male volunteers were dosed orally for 8 days with 300mg FAR or 875/125mg A/C, bid. Oropharyngeal swabs and faeces were collected prior to the 1st dose, and then on 1, 7, and 14 days after dosing. Species representing the oral/faecal flora were sought including *Streptococcus* spp., Lactobacilli, Enterobacteriaceae, Peptostreptococci, enterococci, *Bacteroides* spp., and *Clostridium* spp.. *Clostridium difficile* toxin A + B was analysed by EIA. Colonisation with *Candida albicans*/*krusei* was monitored. Emergence of penem-resistance was screened by using media containing 2, 4, 8mg FAR and 4, 16mg meropenem.

Results: No significant changes in the oropharyngeal flora were seen either in the FAR or the A/C groups. Transient increases in faecal enterococci were seen in both groups, and in *E. coli* in only the A/C group. Counts of *B. fragilis* spp. tended to decrease in both groups. *C. difficile* was not isolated nor toxin detected from any subject. No colonisation with *Candida* spp. was found. Transiently, *P. aeruginosa* was isolated only on day 1, but not 7 + 14d after exposure to FAR (n = 2) or A/C (n = 1). No penem-resistant Enterobacteriaceae were isolated, but in each of 3 volunteers penem-resistant *E. faecalis* were isolated but which disappeared on day 14.

Conclusion: 8 day exposure to FAR resulted in no changes in the oropharyngeal and minimal changes in the faecal flora. *C. difficile* was not selected and overgrowth with *Candida* spp. was not detected. Penem-resistance did not emerge. Thus, FAR does not seem to cause major changes in the resident oro-pharyngeal or faecal flora or lead to the emergence of penem-resistance.

P575 Tifacogin, a recombinant tissue factor pathway inhibitor eliminates serum-resistant *Escherichia coli* from blood cultures

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Objectives: Tifacogin – tissue factor pathway inhibitor (rTFPI), has demonstrated therapeutic effectiveness in acute inflammatory disease after lethal challenge with *Escherichia coli* in several animal models of severe sepsis. Tifacogin is being evaluated in a phase 3 clinical trial in patients with severe community acquired pneumonia. The mechanism of action of tifacogin after acute bacterial infection is not well understood.

Our current study investigates the effects of tifacogin after acute infection in vitro. The objectives were to show whether fragmented tifacogin had antibacterial activity against *E. coli* which could be mapped to the C-terminus and to investigate the effect of heparin on the antibacterial activity of fragmented tifacogin.
Methods: 10% donor derived blood or serum were used to culture 3 strains of serum resistant *E. coli* for 4 hours at 37ºC with varied concentrations of fragmented tifacogin prepared using plasma proteases or synthetic C-terminal peptides. Bacterial survival was assessed by plating serial dilutions of the reaction mixes onto tryptase soy agar plates. Colony numbers were determined after overnight incubation at 37ºC. Direct interaction between TFPI peptides and *E. coli* was investigated by fluorescent microscopy using fixed bacteria and fluorescently labeled peptides. Heparin was included in some cultures or binding experiments.
Results: C-terminal tifacogin fragments and synthetic peptides exhibited antibacterial activity in a complement-dependent fashion. While intact tifacogin slightly enhanced *E. coli* growth, tifacogin digested with plasma proteases dramatically reduced growth. The activity was mapped to a highly positively charged region previously identified within intact TFPI as a heparin binding site. Synthetic peptides including the positive region were highly active (MIC90 10–30nM). Antibacterial action involved the classical complement pathway including terminal complex formation since factors C1q, C2, C3, C4, C5, C6 and C9 were required. Direct interaction with the bacterial cell surface of *E. coli* was seen. Complement mediated killing and cell surface binding were reversed by low amounts of heparin.
Conclusions: Our results show a novel activity of the C-terminus of tifacogin in complement resistant *E. coli* infection that is released by fragmentation of tifacogin. As tifacogin is rapidly degraded in vivo, our findings suggest that tifacogin may have a therapeutic role in management of disease caused by acute infection.

P576 Highlights on the mode of action of ST-246, an anti-orthopoxviral compound

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Objectives: ST-246 is a highly potent and orally bioavailable anti-orthopoxviral molecule that targets the F13L protein of vaccinia virus (VACV). We investigated part of its mechanism of action by using small interfering RNAs, i.e., siF13Ls, designed to specifically knockdown the F13L gene and by characterising ST-246 resistant strains of VACV and camelpox virus (CMLV).
Methods: The VACV strains Copenhagen, Western Reserve (WR) expressing GFP as a C-terminal fusion protein with F13L or an F13L deleted virus expressing GFP from the F13L locus, and the Iran CMLV strain were used. Cultures of human embryonic lung fibroblasts (HEL) were transfected with the siF13L of interest, and infected 24h later at a MOI of 0.01. The effects of siRNA were determined by real-time RT-PCR, viral titrations and flow cytometry. For the selection of resistant viruses, VACV and CMLV were propagated in HEL cells under increasing concentrations of ST-246 for approximately 17 passages. Strains exhibiting at least 100-fold increase of their wild type 50% inhibitory concentration (IC50) were defined as resistant.
Results: Real-time RT-PCR and viral titrations data indicated that siF13Ls (100 nM) knocked down the VACV and CMLV F13L gene transcripts by more than 85% and reduced their replication by 90%. At lower siF13L concentrations, VACV and CMLV replication was inhibited in a different way, which is in agreement with our previously published data on the anti-VACV and -CMLV activity of ST-246. Flow cytometry analysis showed a reduction of VACV-WR replication expressing a F13L-GFP, but not of a F13L deleted VACV-WR, by siF13Ls and ST-246 and validated a common target, i.e., F13L. Furthermore, the IC50 values of two ST-246 resistant CMLV mutants were >20 μg/ml compared to 0.005 μg/ml for the wild type. In the case of VACV, a 120-fold increase of the IC50 value indicated a ST-246 resistant phenotype. Sequencing of the F13L genes, for both resistant and wild type VACV and CMLV strains, is currently ongoing.
Conclusion: We have shown the inhibitory potency of siF13L inducing a specific silencing of the F13L gene which resulted in the inhibition of VACV and CMLV yields. We demonstrated the mode of action of ST-246 at the protein level and not on mRNA. A single target for ST-246 has been validated in VACV and CMLV. We also isolated ST-246 resistant clones of both viruses and the determination of mutations induced by ST-246 in the F13L gene is currently investigated.

P577 REP3123, a novel inhibitor of methionyl tRNA synthetase from *C. difficile*

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Objectives: *C. difficile*-associated disease (CDAD) is caused by overgrowth of toxin-producing strains of *C. difficile* (CD) following disruption of normal gut flora. Currently, there are few therapeutic options and clinical problems are associated with disease recurrence and the persistence of spores in the environment. In this report, we describe biochemical, crystallographic, microbiological and in vivo efficacy properties of REP3123, a novel diaryldiamine inhibitor of CD methionyl tRNA synthetase (MetRS).
Methods: REP3123 was synthesised and purified as a single active enantiomer. CD MetRS was expressed in *E. coli* and purified to homogeneity. The enzyme was crystallised by sitting drop vapor diffusion and structures were solved by molecular replacement. Microbiological testing was done according to CLSI guidelines. Toxins A and B were detected by semi-quantitative immunoassays, Western blot and cytotoxicity assays. In vivo efficacy of REP3123 was evaluated in the hamster model of CDAD.
Results: The tight binding of REP3123 to MetRS (Ki=20 pM) is competitive with methionine but cooperative with ATP. Inhibitor binding is accompanied with a conformational change in the enzyme in which the inhibitor occupies the methionine binding pocket and a newly created hydrophobic pocket. REP3123 is highly active against CD, including the NAP1/027 outbreak strain (MIC range, 0.25–1.0 mg/L). In contrast, REP3123 is inactive against Gram-negative bacteria and many anaerobic bacteria that constitute normal gut flora such as *Actinomyces*, *Bacteroides*, *Bifidobacterium* and *Lactobacillus* species. As a protein synthesis inhibitor, REP3123 inhibits the production of CD toxins A and B under a variety of culture conditions. REP3123 is also highly effective in inhibiting spore formation. In vivo, REP3123 exhibits low oral bioavailability and superior efficacy to vancomycin in the hamster model of CDAD.
Conclusion: REP3123 is a novel mechanism-of-action antibacterial agent that exhibits potent and selective inhibition of CD with limited potential for disruption of normal gut flora. REP3123 effectively inhibits toxin production and sporulation, exhibits low oral bioavailability and protects against CDAD in vivo. With these unique features, REP3123 represents a promising new development candidate for the treatment of CDAD.

P578 Antimicrobial activity of ceftobiprole tested against leading European bacterial pathogens: results from an international surveillance programme (2005–2006)

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Objectives: To evaluate potency of ceftobiprole (BPR) against the most serious and commonly occurring Gram-positive and -negative pathogens isolated in Europe. BPR, an investigational parenteral cephalosporin, is currently under regulatory review following completion of Phase III clinical trials. This agent is uniquely active against oxacillin-resistant (OXA-R) *S. aureus* (MRSA), as well as other Gram-positive and -negative pathogens, making it an attractive candidate for broad-spectrum therapy.

Methods: Consecutive, non-duplicate isolates (17,206) from bloodstream, skin and skin structure and respiratory tract infections were collected from medical centres in Europe (23), Turkey (2) and Israel (1) participating in the BPR Surveillance Program during 2005–2006. Identifications were confirmed by the central monitoring laboratory and all isolates were susceptibility (S) tested using CLSI methods against BPR and comparator agents.

Results: Results are in the Table. Among SA (27% OXA-R) and CoNS (75% OXA-R) isolates tested, BPR inhibited 100% at ⩽4 and ⩽8 mg/L, respectively. While BPR MIC90 values for OXA-R strains were elevated over those of OXA-S strains (8-fold), MIC90 values for other cephalosporins correspondingly increased ⩾32-fold. BPR was 4-fold more potent when testing beta-haemolytic streptococci (BHS) and SPN compared with CRO or FEP; all BHS were inhibited at ⩽0.25 mg/L and >99% of SPN by 0.5 mg/L. BPR was similar in potency to CAZ and FEP (MIC50 values, ⩽1 mg/L) against tested Enterobacteriaceae; coverage against EC was nearly identical for the three agents (94–95% inhibited at ⩽4 mg/L). FEP provided enhanced coverage against KSP (90% at ⩽8 mg/L vs. 78–84% for BPR and CAZ), although BPR and FEP had lower MIC values than CAZ against ESP. Cephalosporins were largely inactive against ESBL-producing EC and KSP. BPR was equal in potency to CAZ (MIC50, 2 mg/L) against PSA and two-fold more potent than FEP, although % inhibited for these agents at ⩽2/4/8 mg/L were similar. None of these agents inhibited >49% of ASP at 8 mg/L.

Species (no. tested)	MIC_{90} (% at ⩽2/4/8 mg/L)		
	BPR	CRO[a] or CAZ[b]	FEP
S. aureus (SA; 4,028)	1 (>99/100/–)	>32 (39/72/75)[a]	>16 (66/76/81)
Coagulase-negative staphylococcus (CoNS; 1,840)	2 (93/>99/100)	>32 (22/33/47)[a]	>16 (41/62/77)
S. pneumoniae (SPN; 1,528)	0.25 (100/–/–)	1 (>99/>99/100)[a]	1 (>99/>99/>99)
E. coli (EC; 2,779)	0.12 (93/94/94)	⩽1 (93/94/95)[b]	0.25 (94/95/96)
Klebsiella spp. (KSP; 883)	>8 (77/78/78)	>16 (80/81/84)[b]	16 (84/87/90)
Enterobacter spp. (ESP; 571)	>8 (81/84/87)	>16 (66/68/70)[b]	4 (88/92/96)
P. aeruginosa (PSA; 984)	>8 (54/65/79)	>16 (56/69/76)[b]	16 (49/66/80)
Acinetobacter spp. (ASP; 320)	>8 (41/41/42)	>16 (15/32/39)[b]	>16 (26/37/49)

Conclusions: BPR displays prominent activity against European staphylococci, including OXA-R strains. The compound also displayed activity against Enterobacteriaceae, similar to that of extended-spectrum cephalosporins, as well as against some non-fermentative bacilli. Given the breadth of its spectrum, BPR may be useful in those European institutions/ regions where MRSA and PSA are both prevalent.

P579 Antimycobacterial activity of two *Berberis* species used as traditional medicine in Iran

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Objectives: Tuberculosis (TB) is the leading cause of mortality worldwide, infecting about 9 million people and killing approximately 2 million people annually. In recent years, emerging multiple drug resistance (MDR) has become a major threat, thus there is an urgent need to search natural products for developing new, effective and affordable anti-TB drugs. Berberidaceae, especially berberis species are well known medicinal plants and are used in traditional medicine, foods and dies in Iran. Chemical composition of Berberidaceae species show that their anti-inflammatory and anti-tuberculosis activity is mainly due to alkaloid constituents. This study was conducted to compare antimycobacterial activity of extracted alkaloids from root bark and fruit of *Berberis vulgaris* and *B. integgerima* from the natural flora of Khorasan ecological region.

Methods: Root bark and fruit from two species were collected, shade air dried, lyophilised and powdered. Chloroform extracts containing alkaloid fractions were obtained. Fresh solutions of each extract were dissolved in 2% DMSO. The Chloroform extracts were incorporated into the Lowenstein-Johnson medium after solidification with final concentrations of 500, 250, 125, 62.5 and 31 μg/ml. Bacterial suspension test was adjusted to 3×10^5 CFU per ml *Mycobacterium bovis* BCG (Strain 1173 P2, Institut Pasteur, Paris, France) and incubated in 37 degrees of Celsius for 21 days. The minimum inhibitory concentration (MIC) amounts were evaluated after 21, 28 and 35 days of incubation. *Mycobacterium bovis* BCG was cultured in presence and absence of reference drug (Kanamycin) as controls. All tests were carried out five times.

Results: All four root bark and fruit chloroform extracts showed significant ($P < 0.05$) MIC concentrations (31–500 μg/ml), therefore 31 μg/ml was considered to be the best MIC for all four extracts.

Conclusion: Medicinal plants are an important resource to find original active drugs or new therapeutic agents especially against TB. The results indicate that root bark and fruit of *Berberis vulgaris* and *B. integgerima* can be used as antimycobacterial agents because of their considerable MIC values. Our investigations show that these remarkable results are due to alkaloids such as berberine, palmatine, oxyacantine and jatrorrhizine. Further investigations are required to assess the activities of these compounds against MDR *M. tuberculosis*.

P580 Antistaphylococcal activity of semi-purified fractions from Eleutherine americana

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Introduction: Meticillin-resistant *Staphylococcus aureus* (MRSA) infections are a global health concern due to the severity of the illnesses they may cause, ranging from mild to very severe infections. The numbers of *S. aureus* strains that exhibit antimicrobial resistance properties have increased and there is a potential risk of transmitting antibiotic resistance genes to the human microbiota through foods. Interest in plants with antibacterial properties has revived as a consequence of current problems associated with the use of antibiotics. Preliminary research from our laboratory revealed that the crude extract from Eleutherine americana produced good antibacterial effect on *S. aureus* from foods. The aim of the present study was to examine the activity of semi-purified fractions from this plant extract against meticillin-resistant strains isolated from foods and enterotoxin-producing reference strains.

Methods: Twenty-two MRSA strains isolated from food together with two enterotoxin-producing strains (ATCC 23235 and ATCC 27664) were used in this study. The Minimum inhibitory concentration (MIC) was determined by the broth dilution method and Minimum bactericidal concentration (MBC) was performed with the extracts that gave significant MIC values using a sterile loop streaking on fresh MHA (CLSI standard methods). Time-kill assay in the presence of these fractions were carried out. The crude hexane extract was chromatographed on column chromatography and fractions were obtained on the basis of their TLC characteristics.

Results: Fraction Ea 6.3 had MIC90 of 0.25 mg/ml while Ea 9.0 gave MIC50 of 0.125 mg/ml with MBC values ranging from 0.25 ⩾ 1.00 mg/ml for the two fractions on all the strains tested. Kill-curve in the presence of Ea 6.3 at 4MIC resulted in total killing of the cells at 20 h for reference strains and 24h for MRSA while fraction Ea 9.0 reduced the inoculum size by 7 log cycle.

Conclusion: Both fractions were very effective against all food isolated MRSA as well as enterotoxin-producing strains. These semi-purified fractions are being investigated in our laboratory to provide alternate treatment against MRSA.

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P581 Approach to identify novel antimicrobials against staphylococci

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Objectives: Bacteriophage endolysins degrade the host cell wall peptidoglycan with near-specie specificity, and thus are of interest as

antimicrobials against *Staphylococcus aureus*. Previous *S. aureus* phage endolysin studies have been based on organisms from Western Europe and North America. Our study extends this to phages from Russia. We have developed a screening system to identify novel phage endolysin proteins of *S. aureus*.
Methods and Results: Lytic bacteriophages have been selected from clinical and environmental samples, or by induction of prophages from a population of temperate bacteriophages from MRSA and MSSA strains. High titer phage lysates of lytic bacteriophage have been tested by nine PCRs to screen for *S. aureus* phages carrying endolysin genes from either five homologous groups (>90%) or four stand alone endolysin genes. This strategy should rule out known endolysin genes and thus identify putative novel genes that are not homologous to previously reported genes. Crude phage lysates harboring candidate novel endolysins are tested for their ability to kill live pathogenic staphylococci in both zymogram analysis and turbidity assays. In the zymogram analysis, selected phage lysates demonstrate two bands (53 kDa and 27 kDa) that are not present in control lysates. Eight phage-associated lysates from candidate novel endolysin harboring phages have demonstrated the ability to kill exponentially growing MRSA and MSSA. Positive isolates are subjected to shotgun cloning of phage DNA and some cloned inserts have been sequenced. Currently, the subcloned phage DNA fragments have no similarity to data deposited in public databases.
Conclusions: The proposed approach is showing promise for identifying novel endolysin genes. Our next focus will be to perform MALDI-TOF analysis on purified phage proteins known to harbor lytic activity in zymogram analysis.
Acknowledgements. This research is supported by ARS-ISTC #499/3571 Project Grant.

P582 Photodynamic therapy against resistant bacteria from cystic fibrosis patients

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Objectives: Photodynamic therapy (PDT) combines a photosensitizer dye with visible light to generate reactive oxygen radicals toxic to cells. High rate of antibiotic resistance in cystic fibrosis (CF) isolates highlights the need for new treatment strategies. This study is aimed to evaluate the in-vitro effect of PDT alone and in the presence of antibiotics on MRSA, *Pseudomonas aeruginosa* (PA), *Stenotrophomonas maltophilia* (SM) and *Burkholderia cepacia* complex (BCc) isolated from CF patients.
Methods: Four MRSA, 4 PA, 8 SM and 2 BCc isolates were initially tested for susceptibility by microdilution method. PDT was applied by using toluidine blue at a light dose of 54 J/cm^2 with a light emitting diode on microtiter plates and killing was determined by viable cell counts. PDT was also applied on antibiotic containing MIC trays and effect on MIC values were tested. MBC values were determined by direct plate count for MIC and MIC+PDT trays.
Results: All PA, Sm and Bcc strains were resistant to ceftazidim (CAZ), meropenem (MEM), ciprofloxacin (CIP) and amikacin (AK), except for two PA strains susceptible to AK and CIP. All MRSA strains were also resistant to AK and CIP. Overall PDT alone exhibited 4 log killing in 8, 2 log in 4, 1 log in 5 and no effect in one strain. PDT application in presence of antibiotics lowered the MIC values in all bacteria except for two PA strains, at least for 4 folds for all the antibiotics tested to susceptible levels. PDT application in the presence of antibiotics decreased viable cell counts at least 3 logs for all the MRSA, SM, BCc strains. PDT+CAZ and PDT+MEM achieved no bactericidal activity in 3 PA strains while AK and CIP exhibited bactericidal activity in the presence of PDT for all PA strains.
Conclusions: Although PDT alone was not able to achieve bactericidal activity for all the tested strains, a significant bactericidal effect was detected when PDT was applied in the presence of antibiotics for all bacteria except for PA. Resistance to antibiotics in CF isolates may be overcome by the synergistic activity of PDT which should further be improved for in-vivo application.

In vitro actibacterial susceptibility studies

P583 Enhanced activity of daptomycin and vancomycin combined with imipenem or oxacillin against community-acquired MRSA isolates

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Objectives: To study glycopeptides and lipopeptides in combination with potent antistaphylococcal β-lactams against community-acquired MRSA (CA-MRSA). CA-MRSA has emerged as a serious clinical therapeutic problem because of limited orally administered treatment options, recognised virulence (Panton-Valentine leukocidin) and ubiquitous presentations in outpatient practice in the USA and worldwide. Serious invasive cases needing hospitalisation require prompt selection of bactericidal, parenteral agents. We report drug combinations with enhanced killing activity.
Methods: Following CLSI susceptibility (S) test screening of >100 CA-MRSA, vancomycin (MIC50, 1 mg/L), daptomycin (MIC50, 0.25 mg/L) and imipenem (IPM; MIC50, 0.5 mg/L) each had potential treatment utility as well as oxacillin (OXA; MIC50, 16 mg/L) in combination. Ten strains with USA300 PFGE patterns (three USA300–0114) or variations were selected for checkerboard synergy/interaction tests. Interpretations used FICA calculations. The control β-lactam was OXA and internal organism control was *S. aureus* ATCC 29213 (MSSA).
Results: All drugs tested alone were consistent with expected results for a MRSA except IPM where contemporary USA300 CA-MRSA MIC values ranged from only 0.03 to 2 mg/L (S levels by CLSI and US-FDA breakpoints). Combinations of IPM with daptomycin and vancomycin clearly indicated enhanced inhibition (and killing; data not shown) for 9–10 of 10 strains (synergy [SYN] or partial SYN [PSYN]). Similarly, OXA with daptomycin or vancomycin demonstrated SYN or PSYN at concentrations easily achievable in vivo. The control MSSA strain interaction results varied from indifference (daptomycin or vancomycin) to PSYN (daptomycin/OXA).

Combination[a]	Interaction category (occurrences):				
	Synergy	Partial synergy	Additive	Indifferent	Antagonism
DAP/OXA	6	4	0	0	0
DAP/IPM	4	6	0	0	0
VAN/OXA	1	9	0	0	0
VAN/IPM	9	0	0	1	0

[a]DAP = daptomycin, OXA = oxacillin, IPM = imipenem and VAN = vancomycin.

Conclusions: Carbapenem (IPM) MIC results for CA-MRSA strains endemic in USA remain low (0.03–2 mg/L; MIC50, 0.5 mg/L) and combinations with either daptomycin or vancomycin demonstrate enhanced inhibition (SYN or PSYN) and bactericidal activity. No antagonism was observed and these combinations should be considered for severe cases of CA-MRSA infections and studies should be expanded to endemic hospital-acquired MRSA.

P584 Activity of oritavancin against *Staphylococcus aureus* isolates that show heterogeneous resistance to vancomycin

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Objectives: Oritavancin is a semi-synthetic glycopeptide that is currently under investigation for use in therapy for severe Gram-positive infections. Its spectrum includes *Staphylococcus aureus* isolates with different vancomycin susceptibility profiles. *S. aureus* isolates heteroresistant to vancomycin (hVISA) are increasingly being reported and have been linked to clinical failure of vancomycin. This study determined the activity of oritavancin against hVISA.
Methods: Clinical isolates that fit the hVISA profile (n = 11) were obtained from the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA) repository and hVISA designations were verified

using (i) teicoplanin screening agar as recommended by the European Antimicrobial Resistance Surveillance System, (ii) the conventional vancomycin and teicoplanin Etest, and (iii) a modified vancomycin and teicoplanin Etest using a higher inoculum and brain heart infusion (BHI) agar plates. MICs were determined by broth microdilution against oritavancin, vancomycin, and teicoplanin according to CLSI guidelines. When testing oritavancin, the procedure was modified to include polysorbate-80 (0.002% throughout test). Plates were read after 24 and 48 h incubation. Reference or quality control strains of different vancomycin susceptibilities (sensitive, intermediate and resistant) were included in all tests as controls.
Results: The hVISA phenotype was confirmed for all isolates. Oritavancin showed potent activity against the hVISAs with an MIC range of 0.25–1 mg/L and an MIC90 of 1 mg/L when the plates are read after 24 h incubation. Oritavancin and comparator MICs read after 48 h of incubation were identical or within one doubling dilution of those read after 24 h of incubation. From the MIC90 values, oritavancin was 2- to 4-fold more potent than vancomycin or teicoplanin.

Agent	MIC range (mg/L)	MIC_{90} (mg/L)
Oritavancin (read after 24 h)	0.25–1	1
Oritavancin (read after 48 h)	0.25–2	2
Vancomycin (read after 24 h)	2–4	4
Vancomycin (read after 48 h)	2–8	8
Teicoplanin (read after 24 h)	2–8	8
Telcoplanin (read after 48 h)	2–16	16

Conclusions: Oritavancin was 4- to 8-fold more potent than the comparator drugs vancomycin and teicoplanin against hVISA strains in broth microdilution assays. This finding supports further development for oritavancin against challenging infections.

P585 Detection of oxacillin resistance in staphylococci: comparison of MicroScan and cefoxitin disc diffusion test

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Objectives: Recently Clinical and Laboratory Standards Institute (CLSI) has recommended the use of the cefoxitin disc diffusion (DD) test for detecting meticillin resistance in Staphylococci. The ability of MicroScan panels PBC 21 (Dade Behring, West Sacramento, California, USA) to detect oxacillin resistance in 786 clinical isolates of Staphylococci was evaluated by comparing results with cefoxitin DD test.
Methods: 425 *S. aureus* and 361 coagulase-negative Staphlylococci (CNS) isolates from all body sites were tested. Microdilution susceptibility testing by MicroScan panel PBC 21 was performed according to manufacturer's instructions. From an 18–24-h blood agar plate, isolated colonies were emulsified in 0.85% sterile saline to yield a turbidity of 0.5 McFarland. The suspension was vortexed, and 0.5 ml was added to 25 ml of sterile distilled water containing 0.02% Tween 80. Panels were inoculated by using the MicroScan inoculator set and incubated for 24 h in MicroScan WA 96.
Cefoxitin DD testing was performed according to CLSI M100-S17 guidelines. The inoculum was prepared directly from 18-h agar plates, and MHA plates (Kima, Italy) were incubated at 35ºC for 24 h. Controls included *S. aureus* ATCC 29213 and ATCC 25923. PBP2a agglutination was performed, as a gold standard, when results of the phenotypic methods were not in agreement. PBP2a agglutination (Oxoid, Cambridge, UK) was performed according to manufacturer's instructions.
Results: 112 (26.3%) strains of *S. aureus* and 231 (64.0%) of CNS isolates were resistant by MicroScan, whereas 114 (26.8%) strains of *S. aureus* and 231 (64.0%) of CNS isolates, respectively, were resistant by cefoxitin DD test. The two strains giving discrepant results between MicroScan and the cefoxitin DD test were examined by PBP2a agglutination and they were confirmed as resistent. The inhibition zone diameters of the discrepant *S. aureus* strains were 22 mm, closed to break-point of resistance.
Conclusion: When coagulase-negative Staphlylococci (CNS) were tested, MicroScan showed an excellent performance to detect oxacillin-resistant Staphylococci, in comparison with the cefoxitin DD test (100% sensitivity). Considering *S. aureus* strains, the sensitivity of Microscan was 98.2%, compared to the cefoxitin DD test.

P586 Tigecycline activity against *Staphylococcus aureus* exhibiting multidrug-resistant phenotypes in Europe

D. Sahm, D. Draghi, C. Pillar, C. Thornsberry, M. Dowzicky (Herndon, Collegeville, US)

Objective: *Staphylococcus aureus* (SA) can cause complicated skin and skin structure infections and complicated intra-abdominal infections. Recently in 2006, tigecycline (TIG) was approved in Europe (EU) to treat these infections. SA worldwide are becoming resistant to the most commonly prescribed antimicrobial treatments. TIG is used in healthcare settings where resistance (R), including multidrug resistance (MDR) has become prevalent. Recent outbreaks of this organism have shown clearly that resistance can develop to several different agents. Careful monitoring of new agents such as TIG has become imperative.
Methods: In total, 686 SA isolates were obtained from 28 hospitals in ten countries across EU during 2006 to 2007. Isolates were tested centrally by broth microdilution (CLSI M7-A7). TIG activity was analysed by oxacillin (OX) and MDR status defined as resistance to $\geqslant$3 agents which included OX, minocycline, erythromycin (ERY), clindamycin (CLI), gentamicin, and levofloxacin (LEV). EUCAST breakpoints were used to interpret TIG MIC results and CLSI (M100-S17) breakpoints were used to interpret all other agents, where applicable.
Results: The susceptibility rate overall for SA tested against TIG was 99.4%, with MICs ranging from 0.03 to 1 mg/L. Regardless of OX or MDR status, the TIG modal MIC and MIC90 (mg/L), remained consistent at 0.12 and 0.25, respectively. TIG maintained the lowest MIC90 of all comparators tested. The most common MDR phenotype was 3-drug R with concurrent R to ERY, LEV, and OX (18.1% of all isolates surveyed) followed by 4-drug R with concurrent R to CLI, ERY, LEV, and OX (17.3% of all isolates surveyed). Among MDR populations, TIG maintained >99% susceptibility rate.
Conclusion: TIG has exhibited exceptional in vitro activity against SA including MDR populations. The potential for R to emerge and spread is increasing among SA according to recent CDC reports, therefore continuous monitoring provided by this surveillance initiative is essential to guide clinicians for new therapeutic options.

P587 Analysis of tigecycline activity against *Staphylococcus aureus* based on patient location and clinical specimen source in Europe

N. Brown, D. Draghi, C. Thornsberry, C. Pillar, D. Sahm, M. Dowzicky (Herndon, Collegeville, US)

Objective: Profiling novel agents such as tigecycline TIG for changing resistance trends is important to determine differences among specific patient populations and sites of infection. TIG, a glycylcycline, was approved in 2006 in Europe (EU) for treatment of complicated skin and skin structure infections and complicated intra-abdominal infections. *Staphylococcus aureus* (SA) has the propensity to develop resistance, which can vary with patient locations (PL) and type of infection (as reflected by varied specimen source [SS]). Therefore, analysis of anti-SA TIG activity was stratified according to PL and SS using data collected during 2006 to 2007.
Methods: SA isolates were collected from locations broadly dispersed across EU (including ten countries) and tested centrally using broth microdilution according to current CLSI standards. TIG activity was analysed by PL (outpatient [OP], intensive-care unit [ICU], and inpatient non-ICU [IP]) and SS (blood [BL], respiratory [RP], skin and skin

structure [SST], and urine [UR]). EUCAST breakpoints were used to interpret all TIG MIC results.
Results: See Table.

Org	Category	TIG			
		Total n	MIC range	MIC_{90}	% Susceptible
SA	OP	109	0.06–1	0.5	99.1
	IP	391	0.03–1	0.25	99.2
	ICU	174	0.03–0.5	0.25	100
	BL	162	0.06–0.5	0.5	100
	RP	242	0.03–0.5	0.25	100
	SST	262	0.03–1	0.25	98.5
	UR	16	0.06–0.5	0.5	100

MIC in mg/L.

Conclusion: Based on susceptibility rates and MIC90s, TIG consistently maintained a high level of activity, regardless of PL or SS. Due to the propensity for SA to develop resistance, continued monitoring of the activity of novel agents against this species is imperative.

P588 Bactericidal activity of iclaprim against meticillin-sensitive and -resistant *Staphylococcus aureus*

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Objectives: Iclaprim is a novel diaminopyrimidine antibiotic that exhibits potent bactericidal activity against major Gram-positive pathogens, notably including MSSA and MRSA. Iclaprim exerts its antibacterial activity by specifically and selectively inhibiting microbial dihydrofolate reductase (DHFR). Intravenous iclaprim recently completed two phase III trials of complicated skin and skin structure infections. This study aimed to determine the bactericidal activity of iclaprim against 16 *S. aureus*, including MSSA and MRSA. Several of these isolates were resistant to the related diaminopyrimidine trimethoprim (TMP).
Methods: MICs were performed against a selection of 16 isolates representative of the MIC range, including MSSA and MRSA, using the CLSI microbroth dilution method. MBCs and time-kill studies were performed under CLSI guidelines.
Results: The MIC range for iclaprim for the 16 isolates of *S. aureus* was 0.015 to 2 ug/ml. The MBC range for all isolates was 0.015–2 ug/ml with MBC / MIC ranging from 1–2. For trimethoprim the MIC range was 0.25–128 ug/ml and the MBC range 0.25–>128 ug/ml. Nine isolates were run in time kill kinetic studies. Iclaprim demonstrated a rapid bactericidal kill with the majority of isolates exhibiting a ⩾ 99.9% reduction in colony forming units following 4.2–7.5 hours exposure to iclaprim. Vancomycin MICs for all isolates ranged from 1–2 ug/ml and MBCs from 1 to >8 ug/ml. The MBC / MIC range was 1–>8. Vancomycin was characteristically slowly bactericidal in time-kill studies.
Conclusions: Iclaprim exhibited rapid bactericidal activity against both MSSA and MRSA, including isolates that were resistant to TMP.

P589 In vitro activity of retapamulin against *Staphylococcus aureus* isolates resistant to fusidic acid and mupirocin

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Objectives: Retapamulin is a novel pleuromutilin antibiotic licensed in the USA in 2007 for the topical treatment of impetigo caused by *S. aureus* and *Streptococcus pyogenes*; It is also licensed in Europe for treatment of impetigo and secondarily infected open wounds caused by the same organisms. We determined its activity in vitro against current *S. aureus* isolates from the UK, including many resistant to fusidic acid and/or highly resistant to mupirocin.
Methods: Six-hundred and sixty four isolates of *S. aureus* were recovered from storage. These had been submitted for reference investigation from laboratories throughout the United Kingdom. MICs were determined on Mueller-Hinton agar in accordance with CLSI guidelines. Susceptibility was categorised using CLSI criteria, where available; otherwise BSAC criteria were used (for mupirocin and fusidic acid).
Results: Susceptibilities of the 664 isolates are summarised below:

Antibiotic	MICs (mg/L)		
	Range	MIC_{50}	MIC_{90}
Retapamulin	⩽0.008–2	0.06	0.125
Mupirocin	⩽0.06–>1024	0.5	1024
Fusidic acid	⩽0.015–>256	2	32
Oxacillin	0.06–>128	128	128
Erythromycin	⩽0.125–>128	128	128
Clindamycin	⩽0.03–>128	0.125	128
Gentamicin	0.015–64	0.5	64

The *S. aureus* included 488 (74%) MRSA (oxacillin MICs >2 mg/L), 336 isolates (51%) resistant to fusidic acid (MICs >1 mg/L), and 254 (38%) with high-level mupirocin resistance (MICs >256 mg/L); 103 (16%) isolates were resistant to both to fusidic acid and to high levels of mupirocin. Retapamulin inhibited 663 (99.9%) isolates at ⩽0.25 mg/L; a single MRSA isolate, also with high level mupirocin resistance required a retapamulin MIC of 2 mg/L.
Conclusions: As a reflection of its unique mode of action, retapamulin has demonstrated excellent activity in vitro vs. *S. aureus*, irrespective of their level of resistance to other antibacterials.

P590 Susceptibility of *Staphylococcus aureus* among skin and wound specimen sources in the United States: laboratory-based surveillance study 2005–2007

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Objectives: As meticillin-resistant *Staphylococcus aureus* (SA) infections increase, current data are needed to assess the susceptibility (S) of SA in the US. In this study rates of antimicrobial S for SA were reported from US laboratories from 2005 to 2007 with regional trends of resistance (R) identified.
Methods: The Surveillance Network (TSN®) comprises 296 laboratories from the 9 census regions of the US. TSN laboratories reported S data for antimicrobials by isolate with specimen source description, region, year of isolation, and patient location (PL including outpatient [OP]; inpatient [IN]; intensive-care unit [ICU]). Commonly prescribed agents were studied, these included ciprofloxacin (CIP), levofloxacin (LEV), clindamycin (CLI), daptomycin (DAP), erythromycin (ERY), gentamicin (GEN), oxacillin (OX), linezolid (LIN), and trimethoprim-sulfamethoxazole (SXT). S data from skin and wound specimen sources were studied during 2005 to 2007. Specific phenotypes were further investigated for associated R and multidrug R (MDR) patterns. Data was interpreted using current CLSI standard criteria.
Results: There were >380,000 isolates of SA tested and reported for the period 2005 to 2007. OX R was observed for 57.0% of SA in 2007 with little change from 2005. There was little difference in rates of OX R between OP and IN cohorts. Among all PL in 2007, R rates (%) were 18.6 for CLI; 64.8 ERY; 37 CIP; 39.5 LEV. Generally as expected, isolates from the ICU tended to be slightly more resistant among the agents studied. Regional variation in R rates was noted with the highest rates in the central regions and lowest in New England and Mid Atlantic regions among the agents studied. There was high activity observed for SXT and GEN. LIN R was extremely rare and DAP R was not observed. MDR rates (%) varied among patient location: 28.5 OP; 39.3 IN; and 51.5 ICU.

Conclusions: SA has become OX-resistant in both the community and hospital setting; however, little change was observed in the past 3 years. MDR strains are now common in all settings and empiric therapy should be guided by local susceptibility patterns due to the regional variation. Currently, SXT, GEN, DAP, and LIN exhibited S rates >95%; however, continued monitoring is warranted due to the propensity of this organism to become resistant.

P591 In vitro effect of fusidic acid on staphylococcus strains with macrolide-lincosamid-streptogramin B resistance

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Objectives: Antimicrobial resistance of staphylococcal isolates continues to be a problem for clinicians worldwide. A major problem in the treatment of *S. aureus* infections is the ability of this pathogen to be resistant to a number of antibiotics. Relationship between meticillin resistance and resistance to other antibiotics has been reported in previous investigations. Fusidic acid displays its antibacterial activity via inhibiting protein synthesis without binding to bacterial ribosomes. This unique mechanism of action prevents cross-resistance between fusidic acid and other antibiotics. This study was carried out in order to determine the in-vitro effect of fusidic acid on stapyhlococcal isolates with macrolid-lincosamide-streptogramine B resistance.
Methods: The study included 532 staphylococcus isolates consisting of 59 MRSA, 101 MSSA, 200 MRCNS and 172 MSCNS. Testing for MLSB and was accomplished by the 'D-zone' test in accordance with the recommendations of the CLSI. Fusidic acid resistance was investigated by agar disk diffusion method according to the criteria of 'Comité de L'antibiogramme de la Société Française de Microbiologie'.
Results: Of 532 staphylococcal isolates, 62.4% were susceptible and 37.6% were resistant to MLSB antibiotics. 62.5% of the resistant isolates exhibited a constitutive resistance phenotype, whereas 37.5% were inducibly resistant. All of 101 MSSA isolates were susceptible to fusidic acid. Susceptibility rate to fusidic acid was higher among strains without MLSB resistance (82.8%) and strains with iMLSB (82.7%) resistance than that observed among isolates with cMLSB (58.4%) resistance. We have also determined that susceptibility to fusidic acid was significantly lower among MRCNS isolates with cMLSB resistance phenotype.
Conclusion: The relatively low susceptibility to fusidic acid detected in our study among strains with MLSB resistance accompanying to meticillin resistance, may be explained with the reports suggesting that multidrug-resistant phenotype can develop as a consequence of continuous exposure of an organism to an antibiotic to which the organism is chromosomally highly resistant. These results indicated that, although fusidic acid can stil be considered as an alternative agent for the treatment of infections caused by meticillin resistant staphylococcal strains, it should be kept in mind that susceptibility rates may be lower than expected if the isolate is also resistant to MLSB antibiotics.

P592 In vitro activity of tigecycline against MRSA, vancomycin-resistant enterococci, and extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* clinical isolates collected in the Czech Republic

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Objectives: To describe the antimicrobial susceptibility to tigecycline in the Czech Republic among isolates of meticillin-resistant *Staphylococcus aureus* (MRSA), extended spectrum β-lactamases (ESBL) producing *Escherichia coli* (n = 90) and *Klebsiella pneumoniae* (n = 100) and vancomycin-resistant enterococci (VRE) (n = 111).
Methods: The isolates were collected in 36 microbiological laboratories partipating in the European Antimicrobial Resistance Surveillance System (EARSS) in the Czech Republic in 2000–2007. The isolates of MRSA (n = 105) and ESBL-producing *K. pneumoniae* (n = 100) were collected from blood. The isolates of ESBL-producing *E. coli* originated from blood (n = 19), urine (11), and wound infections (5). VRE isolates were collected from blood (25), sputum (9), wound (39), urine (34), and stool (5) specimens. A single isolate per patient was accepted. In vitro MICs were determined according to the CLSI (formerly NCCLS) guidelines. The criteria of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) were used to interpret MIC results.
Results: All VRE, MRSA, and ESBL-producing *E. coli* strains were susceptible to tigecycline using EUCAST breakpoints, i.e. ⩽ 0. 25 mg/l, ⩽ 0.5 mg/l and ⩽ 1 mg/l, respectively. As many as 93% of ESBL-producing *K. pneumoniae* strains were inhibited by 1mg/l of tigecycline and five such strains were inhibited by 2 mg/l. Two *K. pneumoniae* strains were resistant to tigecycline with MICs of 4 mg/l.
Conclusion: In the Czech Republic, tigecycline has a good activity against MRSA, VRE, and ESBL-producing isolates of *E. coli*. Strains intermediately resistant (5%) and resistant (2%) to tigecycline were found among ESBL-producing *K. pneumoniae* isolates.

P593 Comparison of daptomycin Etest MICs on Mueller-Hinton, Iso-Sensitest and brain-heart infusion agars with and without calcium supplementation to broth micro-dilution MICs against 20 *Staphylococcus aureus* isolates

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Objectives: The effect of calcium concentration on Etest daptomycin minimum inhibitory concentrations (MICs) using three different types of agar was studied.
Methods: The Etest manufacturer (AB Biodisk) recommends BBL Mueller–Hinton agar (MHA) for daptomycin testing, which historically has calcium levels at 25–30 mg/L. MHA (Mast), Iso-Sensitest agar (ISA, Oxoid) and Brain–Heart Infusion agar (BHIA, Difco) with free calcium concentrations of 8.5–52.9 mg/L were used in this study. Twenty clinical isolates of *Staphylococcus aureus* with daptomycin MICs of 0.25–2 mg/L were selected: these were retested in triplicate using CLSI broth microdilution (BMD; calcium concentration of 52.5 mg/L) and tested once with two lots of Etest on each of the study agars. The quality-control strain, *S. aureus* ATCC 29213, was included with each day's testing. The calcium concentration of each agar and broth was determined using ion selective electrode methodology.
Results: As expected, daptomycin MICs decreased with increasing amounts of calcium (Table). Etest MICs using MHA (calcium 28 mg/L) averaged 1.32-, 1.75- and 1.16-fold higher, whereas unsupplemented ISA averaged 3.3-, 2.9- and 2.3-fold higher than those for strains with BMD MICs of 0.5, 1 and 2 mg/L, respectively. The best correlation of Etest and BMD MICs was with MHA (calcium 42.8 mg/L) and ISA (calcium 52.9 mg/L). Etest MICs on BHIA were significantly higher than BMD MICs, and even with a BHIA calcium concentration of 52.6 mg/L, were approximately one doubling dilution higher than BMD gold standard. There was little lot-to-lot variation noted between Etest MICs.

Media	$[Ca^{2+}]$ mg/L	Mean daptomycin Etest MIC (mg/L) for strains with BMD MICs of:		
		0.5 mg/L (n = 8)	1 mg/L (n = 14)	2 mg/L (n = 14)
MHA[a]	28	0.66	1.75	2.32
MHA	42.8	0.53	1.37	2.06
MHA	51.9	0.31	1.36	1.83
ISA (unsupplemented)	8.5	1.63	2.93	4.67
ISA	30.5	0.85	1.74	2.58
ISA	41.9	0.72	1.42	2.32
ISA	52.9	0.53	1.24	1.98
BHIA (unsupplemented)	21.9	2.17	3.73	5.60
BHIA	30.8	1.93	3.21	4.98
BHIA	42.5	1.38	2.83	4.02
BHIA	52.6	1.07	2.23	3.55

[a]Calcium concentration equivalent to BBL unsupplemented MHA.

Conclusion: This study supports the Etest manufacturer's recommendation that MHA is the best choice for susceptibility testing of daptomycin by Etest. However, there was a large percentage (85.7%) of strains with BMD MICs of 1 mg/L that had Etest MICs of 1.5 or 2 mg/L on MHA (calcium 28 mg/L). The daptomycin susceptible breakpoint is 1 mg/L and there is currently no intermediate or resistant breakpoint. Because of the methodology shift in MIC at the daptomycin breakpoint using Etests on MHA containing 28 mg/L Ca^2+, it is suggested that Etest MICs of 1.5 or 2 mg/L are to be considered indeterminate and retested by BMD. ISA or BHIA media are not recommended for use with the daptomycin Etest strip because falsely resistant interpretation could result.

P594 Confirmation of high-level mupirocin resistance in *S. aureus* isolates using the mupA Evigene™ kit

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Objectives: Mupirocin is the only anti *Staphylococcus aureus* topical antibiotic licensed for eradication treatment of nasal carriage of *S. aureus*. Mupirocin resistance occurs both as "high level" or "low level." Since application of mupirocin ointment into the external nares results in concentrations >20,000 mg/L, only high-level resistance (MIC of >256 mg/L) is significant in this setting. High-level resistance is caused by the expression of the mupA (or ileS-2) gene that encodes an additional isoleucyl tRNA-synthetase that is not bound by mupirocin. In this study we investigated the mupA Evigene™ kit for confirmation of mupA high-level resistance in isolates screened positive using a 5 µg mupirocin disc.
Material and Methods: A total 39 out of 4692 clinical *S. aureus* isolates (1223 were MRSA) with an inhibition zone <20 mm against a 5 µg mupirocin disc were investigated. The 39 isolates were tested by mupA Evigene™ hybridisation assay with capture and detection probes for the mupA gene producing a red colour, assay time of 3.5 hours. Wells were read both visually and using an ELISA reader at 490nm, using an OD of 0.50 as cut off. The isolates were further MIC tested by E-test (using the manufacturer's instructions) and a 200 µg mupirocin disc using confluent inoculum on Mueller Hinton agar. *S. aureus* ATCC 29213 (mupA negative) and AIS2006–079 (mupA positive) were included in all runs.
Results: Six isolates, all MRSA, were found positive for mupA by the mupA Evigene™ both visually and using Elisa reader (OD 2.0–2.7 for positive compared to <0.16 for mupA negative isolates). All 6 mupA positive isolates had MIC >1024 mg/L and inhibition zones <13mm using a 200 mu;g disc, whereas mupA negative isolates had MICs between 0.064 and 8 mg/L and inhibition zones between 20 and 37mm by disk diffusion.
Conclusion: In summary, the mupA Evigene™ identify isolates with high-level resistance to mupirocin fast (3.5 hours) from isolates with low resistance. This investigation supports the use of mupA Evigene™ in clinical settings for the confirmation of high-level resistance in mupirocin resistant *S. aureus*.

P595 Evaluation of the Vitek AST-GP66™ panel and Vitek Two™ for susceptibility testing of selected antibiotics with coagulase negative staphylococci

M. John, J. Daniel, D. Diagre, S. Milburn, B. Wilson, Z. Hussain (London, CA)

Objective: To compare the AST-GP66™ panel and Vitek Two™ with agar dilution for determination of susceptibility of antibiotics commonly used to treat coagulase negative staphylococci (CoNS) infections (clindamycin, erythromycin, linezolid, oxacillin and vancomycin).
Method: Susceptibility was performed on 693 CoNS isolates, comprising 14 species. Panels were set up and tested as per the manufacturer's instructions. Agar dilution was performed using CSLI methods. The Mec A status of isolates was determined using PCR. Agar dilutions covered all the MIC values obtained from the Vitek panels. Differences of ⩾ 2 dilutions were noted. In addition, MIC's were converted to qualitative categories (sensitive or resistant) according to CSLI guidelines. Category errors were then assessed as very major (resistant by Vitek™, sensitive by agar dilution), major (sensitive by Vitek™, resistant by agar dilution) or minor (change from sensitive to intermediate).
Results: In total 3,485 MIC values were generated by each method. 3,443 MIC values were evaluated, out of which 126 had ⩾ 2 dilution differences (3.6%). The remaining pairs differed by ⩽ 1 dilution. Altogether there were 6 very major errors, 22 major errors and 13 minor category errors. For clindamycin there were no very major errors, 7 major and 10 minor category errors. For erythromycin there were 5 very major, 11 major and 2 minor errors. For linezolid there was 1 major error (no very major or minor) and for vancomycin 1 minor (no very major or major). For oxacillin there was 1 very major and 3 major (no minor) category errors (only calculated for *S. epidermidis* and *S. scheifleri*). For other species the MIC did not necessarily correlate with the presence of the MecA gene. The cefoxitin screen (6 µg) did not correlate well with the presence of the Mec A gene.
Conclusion: Vitek Two™ and Vitek AST-GP66™ panels were reliable for determination of susceptibility of CoNS to clindamycin, erythromycin, linezolid, oxacillin and vancomycin. Our results also confirm that CLSI guidelines (presence/absence of MecA)should be followed for interpretation of MIC's between ⩾ 0.5 and 4.0 for oxacillin. The cefoxitin screen was not useful in identifying MecA positive isolates.

P596 Validation of agar dilution for susceptibility testing of coagulase-negative staphylococci to tigecycline

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Objectives: To compare in vitro susceptibilities of a large series of speciated coagulase negative staphylococci (CoNS) to tigecycline using the E-test™ and agar dilution. A secondary objective was to determine whether tigecycline agar dilution plates were stable over a two week period.
Methods: Susceptibility was performed on 693 CoNS isolates, comprising 14 species. E-test has been validated as a method for determining susceptibility to tigecycline and was performed according to the manufacturer's instructions and read in a blinded fashion by two examiners. Agar dilution was performed using methodology described by the CLSI. In addition, a proportion of isolates were re-tested by agar dilution for a period of two weeks to ascertain the stability of tigecycline plates. MIC's were converted to qualitative categories (sensitive or resistant) according to CSLI guidelines. Category errors were then assessed as very major (resistant by E-test™, sensitive by agar dilution) or major (sensitive by E-test™, resistant by agar dilution).
Results: Tigecycline is bacteriostatic against CoNS, which made the determination of MIC's by E-test™ at times difficult to read. Overall, MIC's determined by agar dilution were one dilution higher than those determined by the E-test™. When converted to qualitative categories (sensitive or resistant) this did not result in category changes in most cases when interpreting susceptibility. Overall there were 3 very major category errors (resistant by E-test™, sensitive by agar dilution) and 30 major errors (sensitive by E-test™, resistant by agar dilution). Almost all category errors occurred with *S. haemolyticus* (34%) and *S. epidermidis* (11%). MIC's by agar dilution did not change when the testing was repeated over a two week period.
Conclusion: Apart from for *S. haemolyticus*, agar dilution appears to be a valid method for determining susceptibility of CoNS to tygecycline. In addition, once agar dilution plates have been prepared the tigecycline remains stable for at least 2 weeks at 2–8ºC.

P597 Oritavancin sterilises in vitro biofilms of *Staphylococcus epidermidis* and vancomycin-susceptible and -resistant enterococci

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Objectives: Oritavancin (ORI) is a semi-synthetic lipoglycopeptide that is currently in clinical development for serious Gram-positive

infections. We sought to determine whether ORI can eradicate biofilms of *Staphylococcus epidermidis* and vancomycin-susceptible and -resistant enterococci, which are prominent in infections of indwelling devices and infective endocarditis, respectively.

Methods: The following strains were used for in vitro biofilm studies: vancomycin (VAN)-susceptible *Enterococcus faecalis* ATCC 29212 (VSE), VAN-resistant *E. faecalis* ATCC 51299 (VanB VRE), *E. faecium* ATCC 51559 (VanA VRE), *S. epidermidis* ATCC 12228 and the slime-producing strain *S. epidermidis* ATCC 35984. Biofilms were established in MBEC^{TM} Physiology & Genetics Assay plates (Innovotech; Edmonton, Canada). Minimal biofilm eradication concentrations (MBEC) values for single antimicrobial agents (ORI, VAN, linezolid [LIN]) and for agents in combination (ORI with moxifloxacin [MOX] or rifabutin [RFB]) were determined in three independent experiments.

Results: Biofilms of VSE, VanB VRE and VanA VRE were sterilised by ORI at MBECs of 2 to 4 mg/L following 24 h of drug challenge. In contrast, the VSE and VRE biofilms were tolerant to VAN and LIN, exhibiting MBEC values >128 mg/L for both agents. Biofilms of *S. epidermidis* ATCC 12228 were sterilised by ORI at an MBEC of 2 to 4 mg/L but also exhibited tolerance to VAN and LIN with MBECs >128 mg/L. Sterilisation of *S. epidermidis* ATCC 35984 biofilms required combinations of ORI (MBEC of 4 mg/L) and MOX (MBEC of 4 mg/L), or ORI (MBEC of 4 mg/L) and RFB (MBEC of 0.125 mg/L).

Conclusion: Oritavancin shows promise as monotherapy or in combination against medically important biofilms derived from VSE, VRE and *S. epidermidis* strains.

P598 Prevalence of antimicrobial resistance of enterococcus species isolated from blood patients with haematological malignancies in Russia

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Objective: The aim of this study was to determine the prevalence of enterococcal species isolated from blood cultures and their antibiotic susceptibility in Hematological Centre throughout a 5-year period (January 2003 – November 2007).

Methods: Enterococci were identified by basic tests and by PCR amplification of ddl genes. Minimal Inhibitory Concentrations (MICs) were determined by broth microdilution method (CLSI). Resistance genes were detected by PCR.

Results: A total of 80 enterococcus species were collected from January 2003 to November 2007. Most were *Enterococcus faecium* (65), the rest were *E. faecalis* (14) and E. durans (1). The number of *E. faecium* cases per year increased from 63% (10/16) in 2003 to 86% (18/21) in 2007. *E. faecium* strains (vs. *E. faecalis*) were more resistant to the following antibiotics: levofloxacin (98 vs. 33%), erythromycin (94 vs. 64%), highly-level resistant to streptomycin (89 vs. 43%) and gentamicin (97 vs. 50%); except chloramphenicol (11 vs. 43%) and tetracycline (26 vs. 64%). Five (7.7%) *E. faecium* isolates were resistant to vancomycin (MIC ranged from 128 to 1024 mg/L) and teicoplanin (MIC from 16 to 32 mg/L) and carried vanA genes. *E. faecium* showed an increase in vancomycin resistance from 7% (1/15) in 2005 to 10% (1/10) in 2006 and 17% (3/18) in 2007 and a trend to decrease chloramphenicol resistance from 20% in 2003 to 0% in 2006 and 2007. After long treatment with linezolid, VREF strain reisolated from blood displayed low level linezolid-resistance with a MIC = 4 mg/L.

Conclusion: *E. faecium* was the predominant enterococcal specie isolated from blood cultures. The rate of vancomycin-resistant *E. faecium* bloodstream infections has increased since 2005 to 2007. *E. faecium* was found to be increasingly chloramphenicol sensitive. The occurrence of low level linezolid resistant is a threatening problem for Russian hematological patients.

P599 In vitro activity of tigecycline against enterococci

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Objectives: This study was undertaken to determine in vitro activity of tigecycline, the first representative of glycylcyclines, against 65 enterococci French isolates and to correlate this activity with their resistance gene content. Moreover, the activity of tigecycline was compared to that of other tetracyclines (tetracycline, minocycline, and doxycycline) and 4 main antibiotics used in the treatment of enterococci infections (amoxicillin, vancomycin, linezolide, quinupristine-dalfoppristine (QP)).

Methods: MICs were determined using the CLSI agar dilution method. We studied 55 clinical isolates of enterococcus of human origin and 10 isolates of animal origin (30 *Enterococcus faecalis* and 35 *Enterococcus faecium*) among which 11 strains resistant to glycopeptides (VRE) harbouring the vanA or vanB gene. The presence of the 2 main genes encoding tetracycline resistance in Enterococci: tet (M) (ribosomal protection) and tet (L) (efflux) was detected by PCR on all the strains.

Results: Among the 65 isolates studied, 22, all *E. faecium*, were resistant to amoxicillin (MIC range 0.125–64 mg/L). Only 49% of the strains were susceptible to QP. All the strains were susceptible to linezolide (MIC range 1–2 mg /L). Among tetracyclines antibiotics, tetracycline was the least active molecule with 41% (n = 27) of susceptible strains (MIC90: 64 mg/L); 46% (n = 30) strains were susceptible to doxycycline (MIC90: = 16 mg/L) and 54% (n = 36) to minocycline (CMI90: 64 mg/L). All the 43 tetracycline resistant strains ((MIC > 8 mg /L) possessed at least one of the 2 genes: tet (L) or tet (M). All the studied strains, whatever their phenotype and genotype of tetracyclines resistance, were highly susceptible to tigecycline with MIC90 equal to 0.125 mg/L (MIC range 0.03–0.125 mg/L). No difference of susceptibility was observed according to the species and to the phenotype of resistance.

Conclusion: Tigecycline presents a good in vitro activity against *E. faecalis* and *E. faecium* including VRE. This activity is not modified in the presence of genes tet (M) and tet (L) the most frequent in Enterococci encoding tetracyclines resistance.

P600 Impact of the new CLSI non-meningitis penicillin breakpoints on the reporting of antimicrobial susceptibility in pneumococcal infections

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Objectives: The present breakpoints for defining the susceptibility of meningeal pneumococcal isolates to cefepime, cefotaxime, and ceftriaxone are different from those for nonmeningeal pneumococcal isolates due to the attainability of these agents in the cerebral spinal fluids. However, although the penicillin breakpoints were defined based upon measured penicillin concentrations in the cerebral spinal fluids after intravenous infusions and upon meningitis treatment failures, the breakpoints have been applied to nonmeningeal pneumococcal isolates since the definitions were developed in the late 1970s. In 2008, a new set of breakpoints will be published by the CLSI to be used for defining the penicillin susceptibility of nonmeningeal pneumococcal isolates. The present report aimed to assess the impact of the new breakpoints on the reporting of pneumococcal susceptibilities.

Methods: The MICs of penicillin to pneumococcal isolates were examined by the standard broth microdilution method as recommended by the CLSI. The results between 2000 and June 2007 were collected and analysed against both of the old (susceptible, $\leqslant$ 0.06 mg/L; intermediate, 0.12–1 mg/L; resistant, $\geqslant$ 2 mg/L) and new (susceptible, $\leqslant$ 2 mg/L; intermediate, 4 mg/L; resistant, $\geqslant$ 8 mg/L) definitions.

Results: Results from a total of 3156 pneumococcal isolates, including 1.1% of meningeal isolates, were available for analysis. With the old definition, the rate of penicillin nonsusceptibility increased from 88.6% in 2000 to 92.9% in 2007 (average, 91.2%). With the new definition, the rate of penicillin nonsusceptibility decreased from 34.5% in 2000 to 2.5% in 2007 (average, 20.4%). Notably, the proportion of isolates with

the MICs of 1–2 mg/L increased significantly from 41.4% in 2000 to 70.7% in 2007.
Conclusions: Application of the new breakpoints will result in a significantly lower rate of penicillin nonsusceptibility in nonmeningeal pneumococcal isolates. Clinicians may feel more confident in using penicillin for the treatment of pneumococcal infections, and thus the use of other advanced drug classes may be exempted. However, as the population of pneumococcal isolates with borderline penicillin susceptibility (MIC, 1–2 mg/L) is increasing, the regimen of penicillin treatment must be closely monitored to achieve a satisfied therapeutic outcome.

P601 A longitudinal perspective on *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* resistance trends in Europe: from GLOBAL surveillance studies

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Objective: *Streptococcus pneumoniae* (SP), *Haemophilus influenzae* (HI), and *Moraxella catarrhalis* (MC) are three of the most common pathogens associated with community-acquired pneumonia (CAP). Changes in the prevalence of resistance (R) and multi-drug R (MDR) among these pathogens can become problematic for clinicians treating infections. R rates can vary according to regional distributions. The GLOBAL Surveillance (SUR) initiative is a longitudinal study that benchmarks antimicrobial R among respiratory pathogens (RPs).
Methods: SP, HI, and MC isolates taken from patient specimens were collected from 6 countries in Europe (EU): Belgium (Bel), France (Fr), Germany (Gr), Italy (It), Spain (Sp), and the United Kingdom (UK). The years (Y) of collection were 03–04 (04), 05–06 (06) and 07 (respectively) for SP (N: 3213; 1542; 755); HI (N: 3183; 1578; 860) and MC (N: 722; 379; 163). All isolates were centrally tested by broth microdilution and interpreted according to CLSI standards (M7-A7 M100-S17). Data were analysed according to β-lactamase (BL) status [positive (+) and (−)] for HI and MC; for SP analysis included penicillin (PEN) and MDR phenotypes [R to ⩾ 2 drugs: PEN, cefuroxime, trimeth-sulfa, azithromycin (AZI), and tetracycline].
Results: Overall for SP, PEN-R (%) was 12.7 in 04, 12.8, in 06 and 11.8 in 07. The PEN-R rates (%) varied in 04, 05, and 07, respectively: Bel (7.4; no data; 6.8), Fr (32.9; 23.7; 19.4), Gr (3.5; 5.7; 1.3), It (6.8; 7.7; 21.2), Sp (25.4; 20.8; 7.9), and UK (2.2; 2.8; 3.9). The prevalence (%) of MDR-SP ranged from 3.4 (UK) to 44.2 (Bel) in 04, 4.3 (UK) to 33.2 (Fr) in 06, and 12.4 (UK) to 48.2 (It) in 07. >98% of MDR-SP were susceptible (S) to levofloxacin (LFX). For HI, BL + rates (%) varied by country from 8 (Bel) to 32.3 (Fr) in 04, 7.3 (Gr) to 23.4 (Fr) in 06, and 3 (It) to 18.9 (UK) in 07. Based on MIC90, LFX and ceftriaxone were the most active agents tested against HI, regardless of BL status with MIC90s of 0.015–0.03 mg/L all Y. For MC, overall BL + rates (%) increase by Y slightly: 96.4 (04), 96.6 (06), and 98.2 (07). Regardless of phenotype, LFX and AZI were the most potent agents against MC, based on MIC90s (⩽0.06 mg/L).
Conclusion: LFX showed potent activity against the RPs studied. LFX was active among PEN-R and MDR-SP and BL +/− HI and MC. %S among SP, HI, and MC remained high all Y for LFX. Continued SUR is warranted to monitor any changes that may occur among agents used to treat CAP pathogens.

P602 Antimicrobial resistance in *Streptococcus pneumoniae* and *Haemophilus influenzae* isolated from respiratory tract infections between 2001 and 2007

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Objectives: Local data on antimicrobial resistance is necessary in the empirical therapy of respiratory tract infections. The rate of antimicrobial resistance in respiratory isolates of *Haemophilus influenzae* and *Streptococcus pneumoniae* from children were determined and were compared by year.
Methods: Antimicrobial resistance in respiratory isolates of *H. influenzae*(n = 565) and *S. pneumoniae* (n = 736) were investigated. Susceptibility to penicillin and cefotaxime were determined by E-test (AB Biodisk, Solna, Sweden) in *S. pneumoniae*. In vitro activity of the remaining antimicrobials were evaluated by disk diffusion method according to CLSI criteria. for *H. influenzae*, strains β-lactamase production was detected using chromogenic nitrocefin disk method (BD, Sparks, USA).
Results: Resistance rates are given in the Table. β-lactamase production was detected in 3.4% of *H. influenzae*. Penicilin (intermediate and high-level) and trimethoprim-sulfamethoxazole (TMP/SMX) resistance rates were 34.7% and 44.4% in 2001 and were 55.2% and 60.9% respectively in 2007 for *S. pneumoniae*.

Table: Overall rates of resistance to antimicrobial agents in respiratory *H. influenzae* and *S. pneumoniae* isolates (2001–2007)

	H. influenzae			*S. pneumoniae*		
	n	I (%)	R (%)	n	I (%)	R (%)
Ampicillin	565	–	5.5	ND	ND	ND
Penicillin	ND	ND	ND	736	38.1	4.2
Ampicillin/sulbactam	565	–	0.7	ND	ND	ND
Cefaclor	518	2.9	2.5	ND	ND	ND
Cefotaxime	565	0.2	–	736	–	–
Meropenem	535	–	–	ND	ND	ND
Clarithromycin	557	0.9	2.5	ND	ND	ND
Erythromycin	ND	ND	ND	736	1.1	21.6
TMP/SMX	565	0.4	18.5	736	7.7	45.6
Ofloxacin	509	–	–	736	1.2	0.2
Levofloxacin	ND	ND	ND	460	0.7	–
Tetracycline	514	0.2	0.9	702	2.6	24.5
Chloramphenicol	ND	ND	ND	736		10.7

ND: not determined.

Conclusions: Highest rate of resistance has been observed for trimethoprim-sulfamethoxazole in both microorganisms. β-lactamase production in *H. influenzae* has not increased over the years. Penicillin resistance has increased in *S. pneumoniae*. Resistance to levofloxacin is very low at present. Monitoring of the resistance rates may help the clinicians in the empirical therapy of respiratory infections due to *S. pneumoniae* and *H. influenzae*

P603 Bactericidal activity of moxifloxacin and levofloxacin against *Streptococcus pneumoniae* with dual gyrA and parC mutations

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Objectives: *Streptococcus pneumoniae* (Sp) is the most common bacterial cause of respiratory tract infections (RTIs). RTIs are virtually always treated empirically without the benefit of susceptibility tests. Although fluoroquinolone-resistant Sp isolates are uncommon, these have been associated with clinical failure. Thus, we sought to determine the efficacy of moxifloxacin (MXF) and levofloxacin (LFX) against isolates with double-step parC/gyrA mutations.
Methods: Eight strains of Sp collected via an active surveillance programme were characterised for gyrA and parC QRDR mutations by PCR and DNA sequencing. MICs of MFX and LFX were evaluated using a microbroth dilution method and their bactericidal activity determined using Mueller–Hinton broth supplemented with 5% lysed horse blood in a pharmacodynamic model. Sp were inoculated at a density of 1 x 10^6 CFU/ml, incubated at 35ºC, and examined for viable growth at 0, 1, 2, 4, 6, 12, and 24 h after exposure to MXF or LFX. Drug concentrations approximated epithelial lining fluid (ELF) levels or Cmax of MXF (400 mg) and LFX (500 mg). Protein binding was accounted for and drug elimination over a 24-h dosing interval simulated.

Results: All eight strains in the study had documented gyrA and parC mutations consistent with fluoroquinolone resistance. MICs ranged between 1 and 4 mg/L for MXF and between 8 and 32 mg/L for LFX. At ELF concentrations, MXF was bactericidal against all eight isolates and LFX was bacteriostatic against four strains and had characteristics comparable with the growth control in four strains. At Cmax concentrations, MXF was bactericidal against five strains and bacteriostatic against three strains, LFX had characteristics comparable with controls for all eight strains.
Conclusions: Only MXF consistently achieved bactericidal activity against high level (parC/gyrA) fluoroquinolone-resistant *S. pneumoniae* at either ELF or Cmax concentrations. When treating patients without the benefit of susceptibility testing, MXF has the greatest likelihood of achieving bacterial eradication, even when challenged with fluoroquinolone resistant *S. pneumoniae*.

P604 In vitro activity of tigecycline against contemporary clinical isolates of *Streptococcus pneumoniae*

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Objectives: *Streptococcus pneumoniae* (SP) remains amongst the most prevalent causes of community-acquired respiratory tract infections. Both penicillin and multidrug resistant strains may compromise drug use and ongoing interest in newer antimicrobial agents necessitates ongoing testing of clinical strains. We determined the in vitro activity of tigecycline against blood and respiratory SP isolates.
Methods: Minimum inhibitory concentration (MIC) testing was based on current Clinical and Laboratory Standards Institute procedure by microbroth dilution using 10^5 cfu/ml tested against doubling drug dilutions in appropriate media. Following incubation, the lowest drug concentration preventing growth was the MIC. For mutant prevention concentration (MPC) testing, $\geqslant 10^9$ CFUs were applied to solidified Todd-Hewitt broth containing doubling drug dilution and following incubation, the lowest concentration preventing growth was the MPC.
Results: A total of 174 isolates (47 blood, 127 respiratory) were tested. MIC values were not different for blood versus respiratory isolates. The MIC50/90/100 (mg/L) values were 0.031/0.063/0.063. The MIC range values were 0.008–0.063 mg/L. Against penicillin intermediate and resistant strains, tigecycline MIC values ranged from 0.008–0.063 mg/L. MPC values ranged from 0.063–0.5 mg/L.
Conclusions: Tigecycline was highly active in vitro against contemporary blood and respiratory SP isolates. No isolate had an MIC above 0.063 mg/L and MIC results were not different against penicillin susceptible or resistant strains. MPC values were 0.063–0.5 mg/L. This data suggests tigecycline to be highly active against SP isolates and also demonstrates a low propensity to select for resistance.

P605 The impact of blood in media on the determination of mutant prevention concentration testing of tigecycline against clinical isolates of *Streptococcus pneumoniae*

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Objectives: Tigecycline (Tig) is the first of a new class of compound – glycylcyclines – with potent in vitro activity against penicillin-susceptible and multi-drug resistant strains of *Streptococcus pneumoniae* (SP). The mutant prevention concentration (MPC) defines the drug concentration threshold blocking the growth of resistant bacterial sub-populations present in high density bacterial inocula. We measured the minimum inhibitory concentration (MIC) and MPC values for Tig against SP isolates and compared MPC results on solidified Todd-Hewitt broth (STHB) to those on STHB + 5% sheep red blood cells (SRBC) and to those on tryptic soy agar (TSA) + 5% SRBC.
Methods: For MIC testing, the recommended CLSI procedure was followed: 10^5 cfu/ml tested against doubling drug dilutions in THB with incubation at 35–37 degree C in 5% CO2 for 18–24 hours. For MPC testing, $\geqslant 10^9$ CFUs were added to drug containing agar plates: 1) TSA-5% SHRB, 2) STHB (1.5% agar) and 3) STHB + 5% SRBC incubated as described for 24–48 hours and screened for growth. The lowest concentration preventing growth was the MIC or MPC depending on method.
Results: For SP isolates (including ATCC 49416) MIC50, MIC90 and MIC range values (ug/ml) were 0.016, 0.031 and <0.008–0.31 respectively. MPC50, MPC90 and MPC range values (ug/ml) on STHB were 0.063 (75%), 0.5 and 0.063–0.5 as compared to 8, >8 and 4–>8 on STHB + 5% SRBC and 8, ⩾16, ⩽1–⩾16 on TSA + 5% SRBC. MPC values for SP ATCC 49416 were 0.125 ug/ml on STHB versus 8 ug/ml on STHB + 5% SRBC.
Conclusions: Tig was highly active in vitro against SP with MICs ⩽0.031 ug/ml. MPC values ranged from 0.063–0.5 ug/ml on STHB and were substantially higher when test media contained blood (>90% ⩾4 ug/ml; >37% ⩾8 ug/ml). This data suggests that SP testing of Tig on blood containing media yields falsely elevated values. STHB appears to be a more suitable media for SP MPC testing against Tig. Tig appears to have a low propensity to select for resistance.

P606 Regional trends in telithromycin and azithromycin resistance among *Streptococcus pneumoniae* isolates

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Objectives: This study compared the in vitro activity of telithromycin, the first ketolide developed for clinical use, with that of azithromycin, one of the most commonly used macrolide for treatment of respiratory tract infections, against 198 *Streptococcus pneumoniae* isolates at some North West Italy's hospitals between 2005–2007. Erythromycin-resistance phenotypes were determined to correlate the different bacterial resistant pattern to antimicrobial susceptibility.
Methods: The determination of minimal inhibition concentration (MIC) was carried out using the microdilution broth method according to Clinical and Laboratory Standards Institute (CLSI) with an inoculum of 10^5 cfu/ml and antimicrobial concentrations ranged from 0.03 to 64 mg/L. Minimal bactericidal concentration (MBC) was determined by plating 0.1 ml from the wells showing no visible growth on agar plates and incubating for 18 h. The erythromycin-resistance phenotype was determined by the triple-disk test described by Giovanetti et al. Commercial disks of erythromycin, clindamycin and josamycin were used.

Table. In vitro antimicrobial activity of telithromycin and azithromycin against erythromycin-susceptible and erythromycin-resistant *S. pneumoniae* isolates

Isolates[a]	Drug	Number of strains having MIC (mg/L) equal to:											
		⩾64	32	16	8	4	2	1	0.5	0.25	0.12	0.06	⩽0.03
Erythromycin-susceptible (n = 137)													
	TEL									20		12	105
	AZM								31	51	31	12	12
Erythromycin-resistant (n = 61)													
$cMLS_B$ (n = 26)	TEL							2	4		6	12	2
	AZM	22	2	2									
M (n = 26)	TEL							14	10				2
	AZM	6	18		2								
$iMLS_B$ (n = 9)	TEL										4	1	4
	AZM	9											

[a] 198 *S. pneumoniae* isolates. $cMLS_B$ = constitutive resistance; M = M resistance; $iMLS_B$ = inducible resistance.
AZM = azithromycin; TEL = telithromycin.

Results: 69.2% of the *S. pneumoniae* isolates were erythromycin-susceptible (Ery-S). Among the 30.8% erythromycin-resistant (Ery-R) pneumococci, 42.6% expressed the constitutive MLSB phenotype, 42.6% the M phenotype and 14.8% the inducible MLSB phenotype. Azithromycin showed a high activity against *S. pneumoniae* Ery-S strains, with MIC values ranging from ⩽ 0.03 to 0.5 mg/L. The Ery-R isolates were all resistant to azithromycin (MICs ⩾ 8 mg/L). On the contrary, both Ery-S and Ery-R streptococci isolates were homogeneously susceptible to telithromycin, with MIC values ⩽ 1 mg/L, according to CLSI. Telithromycin and azithromycin MBC values were

generally higher than the corresponding MICs, reflecting the same trend observed for MIC values for all the strains.

Conclusion: The uniform activity of telithromycin against *S. pneumoniae*, including Ery-R isolates, suggests that this ketolide may offer a valuable alternative for treatment of streptococcal infections. Furthermore, in addition to several other reports, even the findings from this regional study, emphasize that an active surveillance of phenotype distribution and antibacterial resistance in pneumococci is essential to guide the effective use of empirical treatment option for streptococcal infections.

P607 Antimicrobial susceptibility of *Lactobacillus* strains used in probiotic products

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Objectives: Antimicrobial susceptibility testing of *Lactobacillus* spp. used in probiotics products is advisable for checking biosafety. In fact probiotics should not carry acquired resistance potentially transferable to endogenous bacteria; moreover, intrinsic resistance could prevent treatment of a infection due to *Lactobacillus* spp. However there is still a lack of agreement on the guidelines of the susceptibility testing of *Lactobacillus* spp. and of the interpretative breakpoints. The aim of this study was to test the susceptibility of *Lactobacillus* sp.using the guidelines of the new 2006 CLSI M45-A document for infrequently isolated or fastidious bacteria.

Methods: We tested the susceptibility to ampicillin, meropenem, vancomycin, gentamycin, erythromycin, clindamycin (recommended by CLSI, 2006) and, also, 2 cephaloporins and 2 fluoroquinolones of 19 *Lactobacillus* isolates from 5 fermented foods, 5 food supplements and 4 vaginal products available in Italy. The strains were isolated using different media under standardised cultivation conditions. The species designation was determined by standardised methods including API kits and PCR. MICs were obtained by broth microdilution according to 2006 CLSI document.

Results: The MIC results were evaluated using CLSI breakpoints to predict clinical success and microbiological breakpoints suggested by FEEDAP Committee (EFSA J., 2006) to distinguish probiotic strains with acquired resistance. All the *Lactobacillus* isolates were β-lactamase negative and susceptible to ampicillin and imipenem by both breakpoints. The isolates of *L. plantarum*, *L. salivarius* and *L. paracasei* confirmed intrinsic-resistance to vancomycin; the other isolates tested had MIC $\leqslant$ 4 mg/L (susceptibility breakpoint). Gentamycin had poor activity against *Lactobacillus* isolates. Atypical resistance to erythromycin occurred in 1 *L. salivarius* isolate. A wide range of MICs was observed for cephalosporins and fluoroquinolones.

Conclusion: The results showed that *Lactobacillus* isolates used in probiotics do not show particular risk of acquired and potentially transferable resistance. However, for *Lactobacillus* isolates involved in serious infections, the marginal susceptibility or resistance to some relevant drugs should be taken into account.

P608 Effects of tylosin and chlortetracycline on the proliferation of antibiotic-resistant bacteria in a simulated river water ecosystem

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Objective: The objective of this study was to examine the effects of varied concentrations of chlortetracycline (CTC) and tylosin tartrate salt (TYL) on the selection of resistance in aerobic bacterial populations in a simulated river water ecosystem.

Methods: Six replicates of a 10-day experiment using river water in continuous flow chemostats were conducted. In each replicate, 1 chemostat served as the control to which no antibiotic was added. In the first two replicates, TYL was added at concentrations of 100 ng/L and 10 μg/L. In replicate 3, TYL was added at concentrations of 10 μg/L and 50,000 μg/L, and in replicates 4 through 6, TYL was added at concentrations of 10 μg/L, 1000 μg/L and 50,000 μg/L. The amount of TYL present in the chemostats from samples taken on days 0, 3, 7 and 10 was quantified with a commercially available competitive enzyme-linked immunosorbent assay. The viable bacterial count was determined by plate count from days 0 through 10 using 1/10-strength LB agars with and without 16 μg/ml of TYL and Columbia CNA agars with and without 16 μg/ml of TYL. The presence of the erm (B) gene in the bacteria within the chemostats was assessed on days 0 through 10 by extracting DNA from a chemostat sample followed by PCR using previously published primers. Results of the TYL replicates were compared to the CTC replicates that were conducted previously.

Results: There was no significant difference among treatment groups in the total bacterial load within the chemostats when assessed on LB agar plates (8–9 log10 CFU/ml). Although the chemostats with higher levels of TYL had lower bacterial loads on Columbia CNA agar and Columbia CNA agar with 16 μg/ml of TYL, there was no difference among chemostats in the proportion of the total bacterial load that grew on CNA agar with 16 μg/ml of TYL. No differences among chemostats were observed when bacteria were grown on LB agar with 16 μg/ml of TYL. The gene erm (B) was detected on many days of the replicates, mainly in the high TYL concentration chemostats.

Conclusions: Low concentrations of TYL in this in vitro experiment did not select for increased levels of resistant bacteria. This is identical to the observation in the CTC experiment. Whereas high doses of CTC did select for tetracycline-resistant bacterial populations, high doses of TYL did not appear to have this effect. These results question the biological significance of low tylosin concentrations observed in the environment.

P609 Establishment of reference substances for the microbiological assay of antibiotics and MIC values determination

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Objectives: In vitro bacterial susceptibility to antibiotics is tested in number of laboratories. However, quality of antimicrobial agents substances applied in Minimal Inhibitory Concentrations assays, especially – their biological activity, is often unknown. Since 2000 year, we have participated in several collaborative trials organised by European Directorate for the Quality of Medicines to assign potency values for candidate European Pharmacopoeia Chemical Reference Substances (CRS) and WHO International Standard used for the microbiological assays of antibiotics. These standards are also the best substances for MIC values determination.

Methods: The following antimicrobial agents were tested: erythromycin, nystatin, colistin, amphotericin B, spiramycin, bacitracin, and rifamycin. The assay was performed as described in Ph. Eur., using diffusion or turbidimetric methods and standard bacterial strains from ATCC recommended by Ph. Eur. for particular agent. Statistical analysis of results was performed with the usage of CombiStas programme.

Results: Erythromycin: 7 labs participated, obtained by us weighted combined estimate was 948 IU/mg (trial established potency = 960 IU/mg. Nystatin: three CRS batches were analysed, obtained by us results (6041, 5446, 5963 IU/mg), respectively corresponded to trials established potency (6030, 5600, 5710 IU/mg). Colistin: 6 labs participated, obtained by us weighted combined estimate was 584143 IU/vial (trial established potency = 580935 IU/vial. Amphotericin B: three CRS batches were analysed, obtained results (993, 987, 990 IU/mg), respectively corresponded to trials established potency (990, 960, 944 IU/mg). Spiramycin: 7 labs participated, obtained by us weighted combined estimate was 4540 IU/mg (trial established potency = 4530 IU/mg). Bacitracin: 7 labs participated, obtained by us weighted combined estimate was 63.1 IU/mg (trial established potency = 62.9 IU/mg). Rifamycin: diffusion method – 6 lab. participated, obtained by us weighted combined estimate was 857 IU/mg (trial established potency = 856 IU/mg; turbidimetric method – 4 labs participated, obtained by us weighted combined estimate was 849 IU/mg (trial established potency = 856 IU/mg).

Conclusions: Participation in collaborative trials showed, that results obtained in our lab – member of OMCL Network, are very good quality

and allowed to establish antimicrobial potency of several reference substances suitable for the MIC values determination.

P610 Tigecycline – in vitro susceptibility of anaerobic bacteria

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Objectives: To determine the in vitro susceptibility of anaerobic and selected mircoaerophilic bacterial species for tigecycline a new glycylcycline anibiotic. Additionally it should be examined if the test results are biased by the medium or the incubation time used.
Methods: Minimal inhibitory concentration (MIC) testing was performed for tigecycline with the epsilon test (Etest®, AB Biodisk, Sweden) as recommended by the manufacturer. Tigecycline stripes with a concentration gradient from 0.016 μg/ml to 256 μg/ml were used. As test media Columbia blood agar at pH 6.8, Columbia blood agar at pH 7.3, Brucella agar and Schaedler agar were used. 297 isolates, representing 89 species were checked. Cultivation was conducted at 37ºC for 48 hrs and 72 hrs, respectively under anaerobic conditions or in the case of microaerophilic bacteria under mircroaerophilic conditions.
Results: All tested species exhibited low MIC values and could be identified as susceptible. But denpendency from the chosen media and growth conditions could be demonstrated. Comparable results were observed with Schaedler agar and Columbia blood agar at pH 7.3. Lowering the pH value of the Columbia blood agar to 6.8 led to different MIC values, Brucella agar again exhibited different MIC values compared with the other media. A reduction of the incubation time from 72hrs to 48hrs resulted in poor growth for several of the fastidioud species and in minor MIC values.
Conclusion: It could be shwon that the results of tigecycline susceptibility testing of anaerobic bacteria are dependent from the solid growth medium, the pH value and the incubation time. Because of the stability and comparibility of the results with Schaedler agar and Columbia blood agar at pH 7.3 it can be recommended to test tigecycline susceptibility on solid agar media at 37ºC with an incubation time of 72hrs, preferably on Schaedler agar. Because of the low MIC values determined for all isolates tigecycline appears to be a promising new agent for the treatment of infections caused by anaerobic bacteria.

P611 Activity of 5 antibiotics including tigecycline and meropenem against anaerobic bacteria: a French multicentre study

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Objectives: The study was deigned to evaluate the activity of tigecycline (TGC), meropenem (MPM), piperacillin–tazobactam (PTZ), metronidazole (MTZ) and clindamycin (CM) against 155 anaerobic bacteria during a survey carried out in 2007 in 4 French university hospitals.
Methods: MIC were determined using E test strips on Brucella blood agar (inoculum McFarland 1).
Results: See the table.

Microorganisms	N	TIG			MERO			PipTaz			METRO			CLIN		
		MIC_{50}	MIC_{90}	%S	MIC_{50}	MIC_{90}	%S	MIC_{50}	MIC_{90}	%S	MIC_{50}	MIC_{90}	%S	MIC_{50}	MIC_{90}	%S
B. fragilis	62	0.19	0.5	98.3	0.25	0.75	98.3	0.38	2	98.3	0.125	0.25	100	0.75	>256	75.8
Other *Bacteroides*	35	0.19	0.5	100	0.19	0.5	100	0.75	8	97.1	0.25	0.5	100	2	>256	51.4
B. fragilis group	97	0.19	0.5	99	0.19	0.75	99	0.5	2	98	0.19	0.5	100	0.75	>256	61.8
Prevotella spp.	23	0.09	0.19	100	0.19	0.25	100	1	3	100	0.25	0.5	100	0.75	64	69.6
Clostridia	81	0.047	0.25	100	0.5	2	97.5	1	8	98.8	0.19	0.5	98.8	1	16	82.7
GPAC	54	0.047	0.09	100	0.03	1.5	100	0.04	0.5	100	0.125	8	88.9	0.125	2	96.2
All anaerobes	155	0.047	0.5	99.3	0.06	2	98.1	0.125	8	98.1	0.125	0.5	96.1	0.125	>256	62.6

N: Number of tested isolates; TIG, tigecycline; MERO, meropenem; PipTaz, piperacillin + Tazobactam; METRO, metronidazole; CLIN, Clindamycin; %S: Percentage of susceptible strains; GPAC: Gram-positive anaerobic cocci.

Conclusions: TGC was the most potent agent against all Gram-positive anaerobes (MIC⩽ 0.5 mg/L). At least 97% of all anaerobic bacteria were inhibited by TGC, MPM, and PTZ. The clindamycin resistance rate remained high (>40%).

P612 Comparative activity of meropenem, imipenem and piperacillin / tazobactam against 1118 bacteria involved in hospital infections: a French multicentre study

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Objective: This study was designed to evaluate the activity of meropenem (MPM), against 1118 non repetitive strains isolated from bacteraemia (55%), pneumonia (29%), peritonitis (12%) and wounds infections (3%), during a survey carried out in 15 French community and university hospitals, in 2006. Susceptibility tests and Minimum Inhibitory Concentrations (MICs) of meropenem (MPM), imipenem (IPM) and piperacillin plus tazobactam (PTZ) were determined by Etest® method (ET) in each centre. Disc diffusion test for MPM and MICs by the agar dilution method (AD) for MPM, IPM and PTZ were determined in specific centres for *E. coli* (EC), others enterobacteriaceae (EB), *Pseudomonas aeruginosa* (PA), aerobic Gram-negative bacilli (AE), *Acinetobacter* (AB), meticillin susceptible *Staphylococcus aureus* (MSSA) and coagulase-negative staphylococci (MSCNS) *Streptococcus pneumoniae* (SP) and anaerobes (AN).
Results: MICs obtained by AD are listed in the table.

	n	MPM			IPM			PTZ		
		%S	MIC_{50}	MIC_{90}	%S	MIC_{50}	MIC_{90}	%S	MIC_{50}	MIC_{90}
EC	149	100	<0.016	<0.016	100	0.05	0.08	90	1.5	8
EB	149	99	0.019	0.05	99	0.11	0.87	85	1.64	14.8
PA	126	95	0.37	3.1	83	1.22	9.1	90	4.5	16
AB	79	98	0.46	1.35	97	0.33	0.96	42	1.41	123
AE	36	83	1	227	83	0.95	227	90	2.5	884
MSSA	145	100	0.09	0.12	100	0.032	0.046	100	0.96	1.84
MSCNS	137	100	0.06	1.1	100	0.013	0.09	100	0.43	0.85
SP	136	100	0.016	0.22	100	<0.016	0.1	100	0.14	3.3
AN	138	100	<0.015	0.5	99	0.048	0.5	92	0.5	16

MIC: Minimum inhibitory concentration; %S: percentage of susceptibility; n: number of tested isolates.

Conclusions: Compared to IPM, MPM displays lower MICs against EB, EC and PA. Except for AE, MICs90 of carbapenems are <4mg/l. Against AB, carbapenems display similar MICs; Compared to carbapenems, PTZ is less active against EB and AB but not PA. Some discrepancies were noted between MICs realised by ET in each centre and MICs realised by AD in specific centre. Especially for aerobics, MICs by ET were frequently higher than MICs by AD.

P613 In vitro activity of ceftobiprole against select Gram-positive and Gram-negative pathogens isolated from Europe in 2006–2007

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Objective: Ceftobiprole (BPR) is a pyrrolidinone-3-ylidene-methyl cephalosporin with activity against a broad spectrum of clinically relevant pathogens, including meticillin-resistant *S. aureus* (MRSA). BPR is intended for use in hospitals against infections where resistant Gram-positive (GP) and Gram-negative (GN) bacteria, particularly MRSA, are suspected. As such, BPR is currently being developed to treat hospital-acquired pneumonia (HAP) and complicated skin and skin structure infections (cSSSI). The current surveillance initiative assessed the in vitro activity of BPR and comparator agents against GP and GN European (EU) clinical isolates from the past year.
Methods: During 2006 and 2007, GP (*S. aureus* [SA], coagulase-negative staphylococci [CoNS], and *S. pneumoniae* [SP]) and GN (Enterobacteriaceae [EN] and *P. aeruginosa* [PA]) clinical isolates were collected from 28 laboratories in 11 EU countries. All isolates were centrally tested by broth microdilution at Eurofins Medinet, Inc. (CLSI; M7–A7).

Results: Against the tested SA, the MICs of BPR were similar to those of vancomycin and linezolid by MIC90 of 1 mg/L and 2 mg/L, respectively. BPR MICs against MRSA were ⩽ 2 mg/L with the exception of 1.3% of MRSA isolates which had BPR MICs of 4 mg/L. Against EN and PA, BPR was similar to cefepime (FEP) by both MIC50/MIC90 (FEP: 0.06/4 mg/L for EN and 4/16 mg/L for PA).

Organism	Phenotype	Total n	BPR MIC (mg/L)			
			Range	Mode	MIC_{50}	MIC_{90}
SA	All	1,010	⩽0.12–4	1	0.5	2
	MSSA	272	⩽0.12–2	0.25	0.25	0.5
	MRSA	738	⩽0.12–4	1	1	2
CoNS	All	378	⩽0.12–4	1	0.5	2
	MS CoNS	114	⩽0.12–1	⩽0.12	⩽0.12	0.25
	MR CoNS	264	⩽0.12–4	1	1	2
SP	All	432	⩽0.002–0.5	0.008	0.008	0.25
	PEN S	335	⩽0.002–0.06	0.008	0.008	0.015
	PEN NS	97	0.004–0.5	0.25	0.25	0.5
EN	All	1,912	⩽0.015–>32	0.03	0.03	16
	CAZ S	1,656	⩽0.015–>32	0.03	0.03	0.12
	CAZ NS	256	0.03–>32	>32	32	>32
PA	All	533	0.06–>32	2	4	16
	CAZ S	421	0.06–>32	2	2	8
	CAZ NS	112	2–>32	>32	16	>32

PEN: penicillin; CAZ: ceftazidime; S: susceptible; NS: non-susceptible.

Conclusion: BPR had potent in vitro activity against the tested GP pathogens, regardless of resistance to meticillin or PEN. BPR was comparable to FEP against both CAZ S and CAZ NS of EN and PA, though MICs of both FEP and BPR were elevated against the CAZ NS isolates. These results show the potential of BPR for the treatment of HAP and cSSSIs. As BPR is intended for use in hospitals where resistance is common, continued surveillance of BPR activity against target pathogens is warranted.

P614 In vitro activity of a mixture of mustard oils (isothiocyanates) against antimicrobial and multidrug-resistant bacteria

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Objectives: A herbal native preparation containing Tropaeoli majoris herba (nasturtium = N) and Armoraciae rusticanae radix (horseradish = H; Angocin® Anti-Infekt N, Repha GmbH, Germany) may be used for treatment of upper respiratory (URTI) and urinary tract infections (UTI). The preparation's antimicrobial efficacy is based on mustard oils (isothiocyanates = ITC), which are cleaved from glucosinolates after oral intake: benzyl-ITC from N, and allyl and phenylethyl-ITC from H. The objective was to assess the antimicrobial activity of a mixture of ITC against antimicrobial and multi-drug resistant bacteria.

Methods: Agar-dilution susceptibility testing was performed. ITC were mixed according to the proportion within the preparation by adding 50% (v/v) benzyl-ITC, 37.9% allyl-ITC, and 12.1% phenylethyl-ITC (all Fluka, Germany). The oil-mixture was dissolved in tryptic soy broth containing 8% Tween 80. Serial dilutions were performed and the mixture was added to Mueller Hinton agar just before casting in order to obtain concentrations ranging from 1% to 0.001%. Bacterial species were tested by use of a multi-point-inoculator (48 isolates in duplicates). Different bacterial species originated from a German surveillance project on antimicrobial resistance and included ESBL-producing *Escherichia coli* (n = 49), ciprofloxacin resistant *E. coli* (53), ESBL-producing *Klebsiella pneumoniae* (49), imipenem, ceftazidime and multi-drug resistant *Pseudomonas aeruginosa* (50), penicillin and macrolide resistant and intermediate resistant *Streptococcus pneumoniae* (43), meticillin resistant *Staphylococcus aureus* (48), vancomycin resistant *Enterococcus faecium* (50). After incubation for 24h at 37ºC, minimum inhibitory concentrations (MIC) were assessed, followed by subculturing to determine minimum bactericidal concentrations (MBC).

Results: MIC90 (% v/v) / MBC90 (% v/v): ESBL-producing *E. coli* 0.015/0.125, ciprofloxacin resistant *E. coli* 0.03/0.25, ESBL-producing *K. pneumoniae* 0.125/0.125, imipenem, ceftazidime and multi-drug resistant *P. aeruginosa* 0.03/0.06, penicillin and macrolide resistant and intermediate resistant *S. pneumoniae* 0.015/0.03, meticillin resistant *S. aureus* 0.004/0.5, vancomycin resistant *E. faecium* 0.015/>1.

Conclusion: The mixture of ITC displays broad activitiy against various antimicrobial and multi-drug resistant bacteria.

Extended-spectrum β-lactamases – Part 1

P615 Increasing number of ESBL-producing *Escherichia coli* during the last three years in Stockholm – A challenge for the clinical laboratory

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Objectives: This study aimed at describing the epidemiologic situation of ESBL-producing *Escherichia coli* (ESBL-EC) in the Stockholm area during 2005 to 2007. The PhenePlate System (PhP) was validated as a screening method used for epidemiological typing of all ESBL-EC isolates and genotypic characterisation of the phylogenetic subgroups of the CTX-M β-lactamase was performed in parallel.

Methods: Clinical isolates of ESBL-EC for the years 2005 (n = 217), 2006 (n = 315) and 2007 (until September, n = 290) were epidemiological typed with the PhP biochemical typing system for *E. coli*. The global congruence between PhP-typing and pulsed-field gel electrophoretic (PFGE) patterns on XbaI digested DNA was examined for a subset of the isolates (n = 56) by the Comparing Partitions computer software. Unique PhP and PFGE-types were defined as isolates with similarity indices above 0.96 and 0.85 (Dice coefficient), respectively. Index of diversity was determined by Simpson index, while the Wallace coefficient was used to evaluate global agreement between the two typing methods.

Results: A steady increase in the prevalence of ESBL-EC for the years 2005–2007 was observed, while the total number of susceptibility tested isolates between the years was comparable. A tendency towards increased multi-resistance was noted. Using the PhP System 28 major clusters (⩾5 isolates of the same PhP-type) were found and two of these clusters have so far been confirmed with PFGE. In the subset of isolates subjected to parallel evaluation of PhP and PFGE greater discriminatory power was observed for PFGE, although not statistically significant. The Wallace coefficient showed that two strains belonging to the same cluster by PFGE had about 83% chance of having the same PhP-type, while conversely strains belonging to the same PhP-type had 74% chance of having the same PFGE-type.

Conclusion: Efficient epidemiological typing of ESBL isolates within a reasonable time-span is of great importance to clinical laboratories. For this purpose PhP, which is a less labour intensive method than PFGE was validated for epidemiological typing of ESBL-EC isolates. PhP-typing was found to be less discriminatory than PFGE, but can probably be used to exclude similarity by PFGE at the 85% cut-off level. Conversely, PhP-clusters should always be confirmed with PFGE. Genotyping revealed that CTX-M-15 and -14 are the most prevalent ESBL-variants in the Stockholm area.

P616 ESBL-producing Enterobacteriaceae in a County of Sweden during 2002–2007

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Objectives: To investigate the occurence of ESBL-producing Enterobacteriaceae in the County of Östergötland, Sweden.

Methods: From January 2002 until August 2007 ESBL-producing strains were collected in the county of Östergötland with 420 000 inhabitants. The ESBL phenotype was confirmed by Etest with ceftazidime, cefotaxime and cefepime, with and without clavulanic acid. PCR amplification was performed to screen for presence of CTX-M genes. CTX-M positive isolates were subjected to DNA sequencing.

Results: A total of 217 clinical isolates, 191 *Escherichia coli*, 24 *Klebsiella pneumoniae*, 1 *Shigella sonnei* and 1 *Citrobacter koseri*, with an ESBL phenotype was found. The isolates mainly came from the urinary tract but also from blood and other sources. Patients identified with ESBL-postive isolates were 6 in 2002 and increased to 39 in 2006. From January until the end of August 2007 32 patients were found. Onehundred ninety-three bacterial isolates were further investigated. The PCR revealed that among *E. coli* 66% belonged to CTX-M group 1, 32% consisted of CTX-M group 9 and more rare was CTX-M group 2. In *K. pneumoniae* 47% belonged to CTX-M group 1 and the other half was conferred to other enzymes. In *S. sonnei* CTX-M group 1 was found and in *C. koseri* CTX-M group 9 was detected. In 4 patients we found both ESBL-producing *E. coli* and *K. pneumoniae*. Eight patients had repeat isolates with ESBL-producing *E. coli* in urine specimens for more than 6 months.
Conclusions: Prevalence of ESBL in clinical specimens is still rare, less than 1%, in our region but the number of patients with ESBL positive isolates increases. CTX-M group 1 is the dominating enzyme group in both *E. coli* and *K. pneumoniae*.

P617 First clonal outbreak of a multi-resistant CTX-M-15 producing *Klebsiella pneumoniae* in Denmark

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Objectives: Increasing frequency of extended spectrum bete-lactamases (ESBL) producing organisms have been reported worldwide, causing major problems in treatment of serious infections, limiting antibiotic therapy to broad-spectrum antibiotics such as carbapenems.
Until the mid-nineties TEM and SHV and their derivates were the predominant β-lactamases, but recently a new group, the CTX-M β-lactamases, have emerged and now seem to be the most widespread ESBLs in many countries. Since the first case reports in *E. coli* from Japan in 1986, the CTX-M enzymes have disseminated worldwide, especially among Enterobacteriaceae.
The rate of antibiotic resistance in the Nordic countries has generally been low; however, recently outbreaks of ESBL-producing organisms have been described from both Norway and Sweden. We here describe the first clonal outbreak of a multi-resistant CTX-M-15 producing *Klebsiella pneumoniae* in Denmark.
Methods: Antibiotic susceptibility testing was done by disc diffusion (Oxoid disc, Isosensitest agar, SRGA breakpoints). ESBL-production was screened for using a cefpodoxime disc; phenotypic confirmation was by the Combi-disc method using Rosco tablets (ceftazidime and cefotaxime +/− clavulanic acid). Identification of ESBL type was done by PCR and sequencing, typing by PFGE using XbaI.
Results: In April 2007 we became aware of two patients with a multi-resistant ESBL-producing *K. pneumoniae* in a medical ward. The isolates were resistant to gentamicin, ciprofloxacin, cephalosporins, trimethoprim/sulfamethoxazole and piperacillin/tazobactam, but susceptible to meropenem and colistin. Soon thereafter we found more isolates, and a systematic screening of patients on the four involved wards, as well as a retrospective search in archives and freezers, identified a total of 36 patients colonised/infected with the strain. Six isolates dating back before the index cases were found, the earliest from January 2006. All isolates had the same or minor variation of the PFGE pattern.
Conclusions: This is the first report of clonal outbreak of a multi-resistant CTX-M-15 producing *K. pneumoniae* in Denmark. The length of time this strain has been able to survive and spread in the hospital environment fully underscores the potential of this and similar CTX-M producing strains to disseminate worldwide.

P618 Predominantly clonal transmission of CTX-M-1 harbouring *K. pneumoniae* in two wards

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Objectives: Resistance against third generation cephalosporins in *K. pneumoniae* is an increasing problem and is mainly due to extended spectrum β-lactamases (ESBL). A worldwide increase of CTX-M-type β-lactamases has been described in recent years but epidemiologic data from Germany are scarce.
Methods: From January 2006 to April 2007 we collected 38 non-copy isolates of *K. pneumoniae* with an ESBL phenotype from ward A (N = 29) and B (N = 9), located in different hospitals and representing different patient populations. Typing was performed by PFGE after enzymatic DNA macrorestriction with XbaI. PCR for TEM, SHV and CTX-M was conducted in all isolates. In single representative isolates genes coding for CTX-M were sequenced. MICs of β-lactam-antibiotics were determined by Etest or microdilution, susceptibility testing for other antibiotic classes was done by agardiffusion.
Results: 15 and 3 isolates from ward A belonged to clone 1 and 2, respectively. 5 isolates from ward B (clone 3) had identical PFGE patterns. All isolates had an SHV β-lactamase as expected. TEM was found in 21 isolates, a CTX-M β-lactamase was found in 36 isolates. In 34 isolates, including all clone 1, clone 2 and clone 3 isolates, a CTX-M-1 group β-lactamase was found. A CTX-M-9 group β-lactamase was found in two isolates.
MIC50 was >256 mg/l for piperacillin, 8 mg/l for piperacillin/tazobactam, 6 mg/l for temocillin, >256 mg/l for cefotaxim and 128 mg/l for ceftazidim. Resistance rates against other antibiotic classes were 94.7% for ciprofloxacin, 78.9% for tobramycin, 34.2% for gentamicin, 26.3% for doxycyclin and 52.6% for sulfamethoxazol-trimethoprim.
Conclusion: Clonal transmission was the most important factor responsible for spread of CTX-M-1 group harbouring *K. pneumoniae* in both wards. Predominant clones were different in both locations highlighting the significance of local epidemiological studies. Temocillin and piperacillin/tazobactam retained good activity in vitro agains ESBL *K. pneumoniae* isolates in our area.

P619 Epidemiology and molecular screening of ESBL-producing Enterobacteriaceae in a Dutch hospital

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Objectives: Over the last five years, resistance against third-generation cephalosporins has increased exponentially in the Netherlands. Until 2002, ESBL-producing micro-organisms were virtually absent in our hospital. Nowadays approximately 6–7% of clinical *Escherichia coli*, *Enterobacter cloacae* and *Klebsiella pneumoniae* isolates carry plasmids encoding the ESBL phenotype.
In analogy to our MRSA policy, we actively search for ESBL positive micro-organisms to contain their further spread. To enable rapid screening, we developed a real time PCR test to detect the CTX-M gene in Enterobacteriaceae with an increased MIC to third-generation cephalosporins. Theoretically, the benefit of this molecular screening would be a two days shortening in the time needed to detect an ESBL.
Methods: We performed a retrospective analysis of the epidemiology of ESBL-producing *E. coli*, *E. cloacae* and *K. pneumoniae* strains in our hospital. Subsequently, all ESBL-producing strains were screened for the presence of TEM, SHV and CTX-M genes by a classic multiplex PCR. A real-time PCR, detecting all 5 CTX-M groups, was then developed and used to enable rapid, weekly screening for ESBLs on our intensive care unit (ICU).
Results: In 2002, less than 1% of our clinical *E. coli* isolates was ESBL positive. This number rose steadily to 6% in 2007. For *E. cloacae* and *K. pneumoniae*, these numbers were 1.3% in 2002 and 7% in 2007 and 1% in 2002 and 5.8% in 2002, respectively.

Molecular analyses showed that approximately 60% of the ESBL-producing strains in our hospital carried the CTX-M gene, with the relative contribution of CTX-M increasing over time. We therefore developed a real-time PCR to detect the presence of this gene. This PCR was then used in an ongoing weekly screening for ESBL-producing Enterobacteriaceae in our ICU.
Conclusion: The occurrence of ESBL-producing enterobacteriaceae in our hospital has increased from approximately 1% in 2002 to 7% in 2007. Sixty percent of these bacteria carried the CTX-M gene. Molecular screening for this gene in clinical isolates from the ICU speeds up the detection of more than 60% of ESBL-producing enterobacteriaceae in our hospital with two days. Parameters to evaluate the additional value of this newly developed strategy are the reduction of secundary cases and the time needed to control an ESBL outbreak.

P620 Extended-spectrum β-lactamases in the Czech Republic

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Objectives: Extended-spectrum β-lactamases (ESBLs) are one of the most important mechanisms of resistance to newer generation β-lactams in Gram-negative bacteria. In the Czech Republic, there has not been done yet any molecular epidemiology study on ESBL-producing organisms. The objective of this analysis is to characterise ESBLs and ESBL producers in one of the biggest Czech hospitals, the University Hospital in Plzen.
Methods: In 2006, a 1-month survey of ESBL-producing bacteria was performed. Twenty-four non-repetitive isolates of *Klebsiella pneumoniae* (n = 10), *Escherichia coli* (n = 9), *Providencia stuartii* (n = 3), *Enterobacter cloacae* (n = 1) and *Proteus mirabilis* (n = 1) were collected. The isolates were typed by RAPD and used in conjugative transfer experiments for ESBL-mediated resistance. ESBLs were characterised by isoelectric focusing, bioassay, and PCR and sequencing of bla genes.
Results: At least seven different ESBL variants were identified in the isolates. SHV enzymes dominated in *K. pneumoniae* (SHV-5, SHV-12 and probably SHV-2), whereas CTX-M β-lactamases, mostly CTX-M-15, were predominant among *E. coli*. The CTX-M-15-encoding gene was preceded by the ISEcp1 element at a distance of 48 bp. TEM-type ESBLs were produced by the remaining strains; TEM-92 by all *P. stuartii* isolates, and TEM-132 – by *E. cloacae*. Genes coding for SHV-5, SHV-12, CTX-M-15 and TEM-132 were located on conjugative plasmids. A significant number of isolates (*K. pneumoniae* SHV-5, *E. coli* CTX-M-15 and *P. stuartii* TEM-92) appeared owing to clonal dissemination of producer strains.
Conclusions: This work showed a high diversity of ESBL variants in the Czech hospital, which included both globally-disseminated and more locale-specific enzymes.
This work was partially supported by a research project MSM 0021620819.

P621 Monitoring, epidemiology and molecular analysis of extended-spectrum β-lactamase producing *Klebsiella pneumoniae* strains isolated at a large hospital in Budapest during a four-month period

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Objectives: Comprehensive multilevel investigation of outbreaks caused by extended-spectrum β-lactamase producing *Klebsiella pneumoniae* (ESBL-KP) in a large tertiary hospital in Budapest during period of four months.
Methods: During the study period ESBL-KP strains were recovered from 18 different hospital wards. Altogether 270 samples were examined, of these 48 environmental and 163 patient and staff samples, respectively. Antimicrobial susceptibility testing was performed by disk diffusion method according to the recommendations of the CLSI. On the basis of preliminary phage typing results ESBL-KP isolates were tested by PFGE. Molecular typing was performed by SHV, CTX-M and TEM PCRs, plasmid profile analysis, transfer of resistance determinants and sequencing of resistance genes.
Results: 132 ESBL-producing clinical isolates of Gram-negative pathogens (GNPs) were collected from October 2006 to January 2007 from 82 patients and hospital staff as follow: *Klebsiella pneumoniae* (n = 86), *Escherichia coli* (EC) (n = 35) and other species (n = 11). One of 48 enviromental samples was positive for ESBL-KP.
70% of samples recovered from the Neonatal Intensive Care Unit (NICU), 50% of samples from the Neurosurgery and the Chronic Internal Medicine Ward (CIMW), respectively, and 30% of samples from the Intensive Care Unit (ICU) proved ESBL-producers.
The molecular characterisation of ESBL-KP strains isolated from the NICU showed that all of them belonged to the same pulsotype (X clone) and harboured blaSHV-5 gene on plasmid of app. 90 kb.
PFGE analysis of ESBL-KP strains isolated from 9 adult wards revealed parallel existence of three different genetic clones: the Hungarian Epidemic Clone (HEC, pulsotype N), the Epidemic clone III (EC III, pulsotype S) and the clone L. All of them harboured blaCTX-M-15 on large plasmids ranged from 50 to 90 kb.
Conclusion: Three parallel outbreaks caused by ESBL-KP were detected in the NICU, the ICU and the Neurosurgery from October to December of 2006. Additionally high colonisation rate (32%) with ESBL-KP was found in the CIMW.

P622 Outbreak of CTX-M extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* in a large general hospital Trust in the UK

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Objectives: The aim of this study was to investigate the molecular epidemiology of an outbreak of ESBL-producing *K. pneumoniae* in a large general hospital Trust in the UK.
Methods: Isolation of ESBL-producing Enterobacteriaceae mainly from catheter urines increased from approximately 5 to 40 isolates per month at a large general NHS Trust Hospital. All isolates recovered over a three month period were screened for susceptibility to cefpodoxime by the BSAC disc diffusion method and ESBL production confirmed using cefpodoxime, cefpirome and ceftazidime with and without clavulanate. Eighty-one of these outbreak isolates were subjected to multiplex PCR to detect all blaCTX-M genes. Clonal relationship was studied by PFGE.
Results: The eighty-one ESBL-producing isolates included 62 *K. pneumoniae* and 19 *E. coli*; 60 isolates (74.1%) from the hospital and 21 isolates (25.9%) from the community. More than 95% of these clinical isolates were recovered from urinary specimens. Multiplex PCR screening identified a blaCTX-M gene in 55/62 *K. pneumoniae* isolates (88.7%) and all of them harboured a blaCTX-M group 1. Characterisation of the regions surrounding the blaCTX-M showed that all isolates carried ISEcp1 upstream from the blaCTX-M and 35 had a IS26 element inserted into ISEcp1. PFGE analysis confirmed that all 55 blaCTX-M positive *K. pneumoniae* isolates belonged to a single epidemic clone (>87% similarity), designated strain GK.
Conclusion: This is the first well documented report of a large outbreak of CTX-M-producing *K. pneumoniae* in a major hospital Trust in the UK. We demonstrated that the dissemination of CTX-M-producing strains of *K. pneumoniae* both in the hospital and the community is due mainly to the diffusion of a single epidemic clone. The outbreak highlights the epidemic potential of CTX-M-producing *K. pneumoniae* and the importance of strengthening infection control measures. Further genotyping of CTX-M producing isolates by DHPLC and sequencing will be undertaken and extensive investigations to determine the source of the outbreak are continuing.

P623 **Trends in extendeds-spectrum β-lactamase *Klebsiella pneumoniae* in a tertiary Spanish hospital (1993–2006)**

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Objective: The objective of this study was to characterise the ESBL-*K. pneumoniae* strains isolated in a tertiary hospital during a 14-year period (1993–2006).

Methods: Screening of ESBL was performed by double disk synergy test. All available *K. pneumoniae* strains were typed by PFGE (XbaI). The ESBL characterisation was performed by isoelectric focusing of betalactamases and by PCR of ESBL family. All ESBLs genes of strains causing nosocomial outbreaks were sequenced.

Results: During the study period, *K. pneumoniae* was isolated from clinical samples of 4925 patients admitted to our hospital. Three hundred and seventy three (7.6%) *K. pneumoniae* carried an ESBL, and the majority of them (197/373, 53%) were isolated from ICU patients. Isolates from 290 patients were available for further studies. Sixty-seven different PFGE patterns were found among the 290 isolates studied. Two major outbreaks were observed throughout the study period. The first of them, involving 150 patients, occurred between 1993–1995 and was due to the spread of strains of Kp1 clone. The majority (85%) of Kp1 isolates produced one ESBL and 15% two ESBLs. By sequencing 34% of ESBL-Kp1 were identified as SHV-2 (pI 7.6), 51% as SHV-5 (pI 8.2) and 15% as SHV-2 plus SHV-5 (pIs 7.6 and 8.2 respectively). The second outbreak was due to Kp29 clone and accounted for 53 patients. This epidemic Kp29 strain produced an SHV-2 betalactamase (pI 7.6). The majority (66%) of Kp29 isolates were detected between September 1999 and November 2000. Another eight clusters of ESBL-*K. pneumoniae* (SHV-family) involving 2 to 7 patients each were detected throughout the study period. During 1999–2006 period, 8 unrelated *K. pneumoniae* producing CTX-M-9 family were detected. Only 2 unrelated strains harbouring a TEM-family ESBLs were detected in 1995 and 2003, respectively.

Conclusion: Although the global prevalence of ESBL-*K. pneumoniae* infections in our institution was 7.6%, the annual frequency ranged from 3% to 18%. Two major outbreaks were associated with the dissemination of epidemic strains producing SHV-2 and/or SHV-5 ESBLs. Isolation of strains producing CTX-M-9 or TEM families of ESBLs is infrequent among *K. pneumoniae*.

P624 **Extended- and broad-spectrum β-lactamase-producing *Klebsiella pneumoniae* isolates in 17 clinical health institutions in Portugal participating in the ARSIP Program**

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Objectives: We report phenotypic and genotypic analysis of clinical isolates of *K. pneumoniae* of both community and nosocomial origin recovered in various hospitals and public health institutions of Portugal.

Methods: A total of 212 *K. pneumoniae* strains isolated over six consecutive months of 1999 in 14 hospitals and 3 other public health institutions were tested for susceptibility to 23 antibiotics by using CLSI guidelines. New enzymes and respective transconjugants were also tested for susceptibility. PCR and sequencing were used to screen and identify blaTEM, blaSHV, blaOXA, ampC and blaCTX-M genes. Biochemical characterisation was performed by isoelectric focusing. Genetic relatedness of 131 isolates was studied by PFGE analysis.

Results: Mostly strains were from women, patients >60 years-old and urinary tract infections; 86% were resistant to ampicillin, 29% to trimetoprim-sulphametoxazole, 16% to aminoglycosides and 2% to fluoroquinolones; 16% were multidrugresistant and 15% expressed an ESBL phenotype, although only 13% (27/212) of these were confirmed by genotyping. Indeed, molecular methods identified 11 strains possessing blaESBL-SHV genes (blaSHV-2A, blaSHV-5, blaSHV-12 and blaSHV-55), 9 with blaESBL-TEM (blaTEM-3, blaTEM-10 and blaTEM-24) and 7 with blaGES-1, encoding ESBL enzymes; 9 with blaLEN, 8 with blaOKP, and 160 with blaSHV-1 and blaSHV-type encoding non-ESBL enzymes; and 4 strains hyperexpressing blaSHV-1 genes, that expressed an ESBL phenotype. Only IEF method identified the expression of the blaSHV-type gene in 4 strains. Overall, we detected 26 new enzymes: SHV-60 to SHV-62, SHV-73 to SHV-83, LEN-18 to LEN-21, LEN-23, OKP-A-12 and OKP-B-15 to OKP-B-20. Twenty additional strains isolated during a second period, between March and November 2006, in three of the 14 participating hospitals, contained ESBL-encoding genes whereas none of the strains isolated in the same hospitals in 1999 carried such genes: blaSHV-5, blaSHV12, blaTEM-10, blaTEM-52, blaCTX-M-15, blaCTX-M-32 and blaCTX-M-61. The PFGE analysis showed that strains were diverse, and 25 clusters were defined, the largest including 14 strains of different biological origins, 6 of which expressed GES-1 enzymes. No dominant epidemic clone was detected.

Conclusion: We described the expansion of β-lactamases produced by *K. pneumoniae* in 3 large regions in Portugal, providing evidence that the genotypes of *K. pneumoniae* strains in community and hospital environments is changing.

P625 **Spread of CTX-M extended-spectrum β-lactamases among *Escherichia coli* and *Klebsiella pneumoniae* isolates in Italian long-term care facilities: results of the first nationwide prevalence study**

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Objectives: In the present century CTX-M Extended-Spectrum Beta-Lactamases (ESBLs) have become predominant mainly in *E. coli*, and the producers are increasingly isolated from community patients. Over 60 different CTX-M-type enzymes have been identified from around the world. Reports of Italian isolates with CTX-M ESBLs only began to appear in 2003. The objective of this study was to evaluate the diffusion of CTX-M type β-lactamases among *E. coli* and *K. pneumoniae* clinical isolates recovered from Long-Term Care Facilities (LTCFs) residents across Italy.

Methods: All catheterised inpatients at the 37 facilities involved in the study were surveyed in the same day between Sept. 2006 and Jan. 2007. Susceptibility testing of urinary isolates was performed with VITEK 2 System (Bio-Mérieux). All isolates identified as ESBL producers according to CLSI criteria were tested at first for blaCTX-M alleles by PCR with universal primers. ESBL-CTX-M producers detected by phenotypic and genotypic methods were then investigated by using specific primers for various blaCTX-M groups. The clonal relationships between epidemiologically relevant *E. coli* and *K. pneumoniae* strains were evaluated by PFGE.

Results: A total of 496 Enterobacteriaceae isolates were recovered. The overall ESBL producers rate was 39.7%. All isolates were characterised by co-resistances but all retained susceptibility to carbapenems.

The prevalence rates of *E. coli*, the most frequent uropathogen, and of *K. pneumoniae* were 40.5% and 8.5% respectively. Seventy-two *E. coli* (35.8%) and ten *K. pneumoniae* (23.8%) produced ESBLs, with 75% and 60% respectively producing CTX-M enzymes. CTX-M-1-group ESBLs were the most prevalent in *E. coli* (49/54 strains) but CTX-M-9-group variants were also identified (5/54 strains). *K. pneumoniae* isolates produced CTX-M-1 group enzymes exclusively. The five *E. coli* CTX-M-9-type producers were from 4 LTCFs: two located in Southern Italy and the others in Northern Italy. *E. coli* CTX-M-9-type producers were clustered in three clonal lineages, one of which responsible for an epidemic in a LTCF in Catania.

Conclusions: Our data confirm that LTCFs are reservoirs of MDR pathogens and that CTX-M-type ESBLs are widespread also in these settings. The overall CTX-M producers rate in LTCFs is higher than that reported in the second Italian nationwide survey (2003) for isolates from acute-care hospitals. Our results document the dissemination of CTX-M-9-type genes also in Italian area.

P626 Molecular types of extended-spectrum β-lactamases in *Escherichia coli* strains isolated from community-acquired urinary tract infections in Turkey

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Background: Extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* have been increasingly recognised in the community. The aim of this study was to determine the prevalence of CTX-M, SHV, and TEM types of ESBLs for community-onset ESBL-producing *E. coli* in urinary tract infections (UTIs).

Methods: ESBL-producing *E. coli* strains isolated from adults with community-onset UTIs during two distinct periods in 2004 and 2006 were collected from four centres in different cities of Turkey. The presence of ESBL was established on the basis of CLSI criteria and confirmatory tests. Polymerase chain reaction and DNA sequencing were used to characterise the blaTEM, blaCTX-M and blaSHV genes.

Results: We analysed 54 *E. coli* isolates (18 isolates from 2004 and 36 from 2006–2007). The prevalence of CTX-M, SHV, and TEM were 94.4% (51), 9.3% (5), and 42.6% (23) respectively. Sequence analysis of the blaCTX-M containing isolates revealed that these isolates contained two different blaCTX-M, 2 (3.9%) with blaCTX-M-3, and 49 (96.1%) with blaCTX-M-15. We found 3 different ESBL blaSHV types: blaSHV-12, blaSHV-5, and blaSHV-2. There were 2 blaTEM-116 carrying isolates.

The two isolates carrying TEM-116 had also CTX-M-15, and they belonged to the 2006–2007 period. There were no other significant differences in the two periods regarding the ESBL types. In the table the distribution of the CTX-M, SHV, and TEM ESBL types is shown.

Table: The distribution of ESBL types among the isolates

ESBL types			Number of isolates		
TEM	SHV	CTX-M	2004	2006–07	Total
–	–	CTX-M-15	16	31	47
–	–	CTX-M-3	0	2	2
TEM-116	–	CTX-M-15	0	2	2
–	SHV-5	–	1	0	1
–	SHV-2	–	1	0	1
–	SHV-12	–	0	1	1
2	**3**	**51**	**18**	**36**	**54**

Conclusions: ESBL-producing *E. coli* in non-hospitalised patients seem to be emerging with various types and prevalence in different countries. Our study revealed dissemination of CTX-M type ESBL in *E. coli* strains isolated from community-acquired UTIs in Turkey. CTX-M-15 was the most common enzyme.

In this study in only two of the isolates we found ESBL-type TEM, and they were isolated in 2006. This may indicate that a novel ESBL type (TEM-116) has emerged. A close follow up whether this novel ESBL type disseminates would be appropriate. The coexistence of CTX-M-15 with ESBL-type TEM genes has been observed in two isolates, compatible with similar findings in recent studies.

P627 Rapid emergence and dissemination of blaCTX-M among Enterobacteriaceae in USA medical centres: report from the MYSTIC Program (2007)

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Objective: To characterise extended-spectrum β-lactamase (ESBL) and plasmid-mediated AmpC (pAmpC)-encoding genes among Enterobacteriaceae (ENT) isolates collected from USA medical centres, and to identify plasmid-mediated fluoroquinolone resistance (pFQ-R) encoding genes among ESBL-producing isolates.

Methods: 1,392 ENT isolates were collected from 15 USA medical centres through the MYSTIC Program during 2007 and tested by the CLSI broth microdilution method. ESBL-positive (clavulanate inhibited) strains were tested for the presence of PER, GES, VEB, CTX-M and OXA enzymes with a multiplex PCR approach. ESBL screen positive but not confirmed were screened for pAmpC genes. Isolates were also tested for qnr-type, qepA and aac(6′)-Ib-cr. Primers annealing on genetic structures flanking the resistance encoding genes were used to obtain complete DNA sequences. Amplicons were sequenced in both strands and results analysed.

Results: Among 70 (5.0% of the total) ENT isolates with an ESBL phenotype, blaCTX-M was detected in 28 (40.0%; 24 *E. coli*, 2 *K. pneumoniae*, 1 *P. vulgaris* and 1 *K. oxytoca*). CTX-M-15 (18 strains, 64.3%) and CTX-M-14 (9, 32.1%) were most prevalent; CTX-M-3 was observed in one strain (3.6%). These isolates were collected in 12 centres (80.0% of the participating sites). OXA-encoding genes with ESBL spectrums were identified in 10 isolates, one co-producing CTX-M. Five isolates harbored blaCMY-2 (4 *E. coli* and 1 *K. pneumoniae*), while 4 isolates carried blaFOX-5 (3 *K. pneumoniae* and 1 *K. oxytoca*). Nine ESBL-positive (12.9%) isolates carried qnr genes (2 qnrA, 6 qnrB and 1 qnrS). The qnr genes were found in one isolate carrying each of the β-lactamases: blaFOX-5, blaCMY-2, blaCTX-M-15, blaOXA-2 and blaOXA-10. Only one (3.6%) CTX-M-producing strain carried aac(6′)-Ib-cr. This isolate harbored blaOXA-10 and blaCTX-M-14. Meropenem was active against all blaCTX-M and ESBL-producing strains.

Conclusions: For over a decade CTX-M-producing isolates have been reported as the highly prevalent ESBL resistance mechanism in Europe and Asia. In contrast, these enzymes were considered unusual in the USA. In this 2007 collection of ENT from USA medical centres, at least 2 distinct CTX-M-type enzymes have disseminated among different species and institutions. In addition, the prevalence of pFQ-R encoding genes among β-lactamase-producing isolates was higher than that observed in ENT not carrying these resistance determinants.

P628 Occurrence of blaCTX-M genes in quinolone resistance Enterobacteriaceae isolates from Brazil

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Objectives: To determine the prevalence of plasmid-encoded extended-spectrum β-lactamases (ESBL) among a collection of quinolone resistance Enterobacteriaceae isolates recovered in a community setting from Brazil.

Methods: Two hundred fifty seven non-repetitive nalidixic-acid resistant enterobacterial isolates were collected in a private laboratory from January 2000 to May 2005. The production of ESBL was detected according to Clinical and Laboratory Standards Institute (CLSI) criteria. Beta-lactamase-encoding genes were detected by PCR using universal primers for the TEM, SHV, and CTX-M families. The ESBL isolates were tested for qnrA, qnrB, qnrS and aac(6′)-Ib-cr genes by PCR technique. Conjugation experiments were performed to determine whether the ESBL genes carrying plasmids were self-transferable. Hybridisation experiments were performed with a Southern transfer from recombinant and original plasmids harboring blaCTX-M. Genetic structures surrounding the blaCTX-M genes were analysed by PCR and the genetic relatedness of the isolates determined by pulsed-field gel electrophoresis.

Results: Twenty four (9.3%) ESBL-producing Enterobacteriaceae strains were isolated during the study period, 9 were *Escherichia coli*, 6 *K. pneumoniae*, 5 *Enterobacter cloacae*, 2 *Providencia stuartii*, 1 *Morganella morganii* and 1 *Citrobacter freundii*. PCR and DNA sequencing detected isolates harboring blaCTX-M-2 (n = 13), blaCTX-M-9 (n = 3), blaCTX-M-8 (n = 2), and blaSHV-5 (n = 6). The ESBL-positive isolates were also positive for the blaTEM-1 gene, except one *M. morganii* isolate. Plasmids harboring blaCTX-M were not transferable and ranged from 40 to 180 kb in size. One *K. pneumoniae* isolate co-harbored blaCTX-M-2 and qnrB2 gene and an *E. cloacae* isolate that was qnrA positive harbored blaSHV-5. However, ESBL and qnr genes were found in different plasmids. No aac(6′)-Ib-cr gene was found in ESBL isolates

studied. ISEcp1 was identified upstream of the blaCTX-M-9 and ISCR1 was found in isolates harboring blaCTX-M-2.

Conclusion: The results presented in this study provided additional data about the molecular epidemiology of plasmid-encoded ESBL determinants among enterobacterial isolates recovered in a Brazilian community setting. A large diversity of genotypes was circulating in the community, with a predominance of blaCTX-M-encoding non-conjugative plasmids. A low prevalence of Qnr determinants was found in ESBL-producing isolates recovered from Brazilian community.

P629 Phylogenetic groups of non-clonally related *Klebsiella pneumoniae* clinical isolates with and without extended-spectrum β-lactamases

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Objectives: Phylogenetic groups of *Klebsiella pneumoniae* isolates have been associated with the presence of specific chromosomal β-lactamases and the environmental compartment origin. In this study the population structure and phylogenetic groups of different *K. pneumoniae* isolates with and without extended spectrum β-lactamases (ESBL) recovered from hospitalised and non-hospitalised patients were characterised.

Methods: A total of 102 non-clonally related *K. pneumoniae* strains recovered from 26 outpatients and 76 hospitalised patients were analysed. Isolates distribution was as follows: 66 ESBL-producing isolates (1989–2004) (10 blood, 28 urine, and 28 other samples) and 36 non-ESBL-producing isolates (2005–2007) (14 blood, and 22 urine). Clonal typing was established by XbaI-PFGE. Phylogenetic groups (KpI, KpII, and KpIII) were determined by PCR amplification of the gyrA gene and further analysis of the RFLP pattern obtained with HaeIII and TaqI (Brisse et al. CMI 2004; 10:942–5). Amplification of the chromosomal blaSHV, blaOKP and blaLEN genes was also performed. ESBL characterisation was established by PCR and further sequencing.

Results: Distribution of isolates between different phylogenetic groups was as follows: KpI (81/102), KpII (3/102) and KpIII (18/102). This distribution was not substantially affected when considered different sources and resistance patterns. Most of the susceptible isolates obtained from blood cultures belonged to KpI group (13/14) as well as those obtained from urine samples (18/22). The corresponding values for ESBL producers were as follows: (6/10) and (24/28), respectively. Nevertheless, a clear association was observed with CTX-M-10 producing isolates and KpIII-group ($p < 0.001$, Chi squared test), irrespective of clinical source and patients' origin. KpIII non-ESBL-producing isolates were recovered from outpatients. Most of the KpIII-isolates (15/18) were also positive for blaLEN.

Conclusions: KpI is the most prevalent phylogenetic group in *K. pneumoniae* isolates from clinical origin in both ESBL and non-ESBL producers. However, KpIII phylogenetic group, previously associated with environmental sources, was consistently linked with blaCTX-M-10 and chromosomal blaLEN gene. This association might reinforce the potential environmental origin of these ESBL-producing isolates.

P630 Prevalence of faecal carriage of ESBL-producing Enterobacteriaceae in patients with severe abdominal infections

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Objectives: A Swedish multi-centre study was carried out to determine the aerobic enteric faecal flora in patients with severe abdominal infections during hospitalisation, with a special emphasis on carriage of Enterobacteriaceae producing extended spectrum β-lactamases (ESBL).

Method: Rectal swabs were obtained at admission to hospital, during antibiotic treatment and at least 48h after end of antibiotic treatment. Samples were also obtained during operation and at clinical indications. Cultures were performed on chromogenic agar with antibiotic discs. Antibiotic susceptibility testing was performed using the disk diffusion method according to the Swedish Reference Group for Antibiotics. ESBL-phenotype was confirmed by Etest with cefotaxime, ceftazidime and cefepime, with and without clavulanic acid. The isolates were subjected to PCR for screening of CTX-M genes, using universal CTX-M primers, followed by DNA-sequencing.

Results: Of 129 analysed patients, 14 had isolates of Enterobacteriaceae with an ESBL-phenotype. Twelve patients were colonised by only one species with ESBL-phenotype, one patient had two (*Escherichia coli* and *Klebsiella oxytoca*), and one was colonised by three different species (*E. coli*, *Klebsiella pneumoniae* and *Proteus vulgaris*). Many patients had repeat isolates and several subpopulations were detected during the study period. *E. coli* (n = 8) was the most frequently found species, followed by *K. oxytoca* (n = 4), *Citrobacter* spp. (n = 2), *K. pneumoniae* (n = 2) and *P. vulgaris* (n = 1).

Four of eight patients with *E. coli* had findings of CTX-M genes, belonging to CTX-M group 1 or 9. Multi drug resistance was seen among two of these CTX-M producing isolates. One of two *K. pneumoniae* had a CTX-M enzyme belonging to group 1.

K. oxytoca and *Citrobacter* spp. were found to harbour the chromosomally encoded β-lactamases K1 and Sed1, respectively. In *P. vulgaris* no CTX-M or K1 enzyme was detected.

Conclusions: The prevalence of faecal carriage of isolates with ESBL-phenotype among patients with severe abdominal infections was 10.9%. At admission to hospital 5.4% and 0.8% of the patients had ESBL-producing *E. coli* and *K. pneumoniae*, respectively, in their faecal flora. These data may have implications for antibiotic treatment in abdominal infections.

P631 Presence of extended-spectrum β-lactamase-producing Enterobacteriaceae in the faecal flora of patients from general practice

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Objectives: The aim of this study was to determine the extended-spectrum β-lactamase-producing Enterobacteriaceae (ESBL-E) carriage in community patients' faecal flora and to characterise the detected ESBLs.

Methods: This study was performed at the University Hospital of Liège (Belgium). From March 2007 to June 2007, a total of 284 faecal specimens were collected from 284 patients who consulted their general practitioner. Each sample was homogenised in 1 ml of sterile saline and aliquots were inoculated on three different selective culture media: ChromID ESBL agar (bioMérieux) and MacConkey agar + ceftazidime (2 mg/L) and Drigalski agar + cefotaxime (1.5 mg/L). All the Enterobacteriaceae growing on these media were identified and tested for susceptibility by the VITEK 2 (bioMerieux). The detection of ESBL production was performed by combined double disks (ceftazidime, cefotaxime and cefepime disks alone and a disk containing clavulanic acid). Characterisation of these ESBLs was performed by PCR assays targetting blaTEM, blaSHV, blaCTX-M, the most frequent ESBL genes, followed by amplicon sequencing.

Results: Overall, 53 Enterobacteriaceae were recovered on the selective media from 284 samples (18.7%). Among these, 25 were identified as ESBL producers: 20 *Escherichia coli*, 3 *Proteus mirabilis*, 1 *Serratia fonticola* and 1 *Enterobacter aerogenes*. These 25 ESBLE originated from 20 patients (7.04%). Among the ESBL-*E. coli*, the following ESBLs were found: TEM 19 (n = 1), TEM 52 (n = 4), CTX-M 1 (n = 5) and CTX-M 15/28 (n = 2); 2 amplicons of TEM genes were not correctly sequenced. The ESBL from the *E. aerogenes* was TEM 52 and those from the 3 *P. mirabilis* were TEM 24. For 6 isolates, 5 *E. coli* and 1 *S. fonticola*, PCR did not demonstrate any ESBL gene of type TEM, SHV or CTX-M.

Conclusions: (1) Out of all screened community patients, 7% were found to be colonised in their faecal flora with ESBL-E strains. (2) *E. coli* accounted for the majority of ESBL-E isolates, while *P. mirabilis* and *E. aerogenes* were in a minor proportion. No ESBL-*Klebsiella* sp. was recovered. (3) Various ESBL genes were identified. (4) TEM- and CTX-M-derived enzymes were the most frequently encountered

ESBLs. No SHV-derived enzyme was found. (5) The study shows that antimicrobial resistance among Enterobacteriaceae in the community becomes a reality which should probably be taken into account in treatment recommendations.

P632 High prevalence and diversity of extended-spectrum β-lactamases in faecal *Escherichia coli* isolates of healthy humans

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Objectives: To study the prevalence and genetic characterisation of extended-spectrum beta lactamases (ESBLs) in faecal *Escherichia coli* isolates of healthy humans and to analyse the surrounding regions of ESBL genes and its possible inclusion in integrons.
Methods: 105 faecal samples recovered from healthy humans were seeded on Levine agar plates supplemented with cefotaxime (2 mg/L) and were incubated at 37ºC for 24 h. One colony per sample with *E. coli* morphology were identified and screened for ESBL production by double-disk test. All ESBL-positive *E. coli* isolates were included in this study. Susceptibility testing to 17 antimicrobials was performed by disk-diffusion test and genes encoding CTX-M, SHV and TEM type β-lactamases were analysed by specific PCRs and sequencing. The genetic environment of blaCTX-M genes as well as the presence of integrons and their gene cassette compositions were characterised by PCR and sequencing. The phylogenetic group of ESBL-positive isolates was determined by PCR.
Results: ESBL-producing *E. coli* isolates were detected in 7 of 105 analysed samples (6.6%). The ESBL genes found were the following ones (number of isolates): blaCTX-M-14a (1), blaCTX-M-14b (1), blaCTX-M-1 (2), blaCTX-M-32 (1), blaCTX-M-8 (1), and blaTEM-52 (1). The genetic environment of blaCTX-M genes were as follows: i) ISEcp1 and IS903 surrounding blaCTX-M-14a gene; ii) IS26/ISEcp1 and orf477 surrounding blaCTX-M-1 gene; iii) ISEcp1 and orf477 surrounding blaCTX-M-32 gene; iv) blaCTX-M-14b included into the integron In60 with dfrA16 plus aadA2 gene cassette arrangement in their variable region; v) the surrounding region of blaCTX-M-8 gene could not be identified. The blaCTX-M-32-positive isolate harboured a class 1 integron which included a variable region of 2000 bp. The phylogenetic groups A, B1 and B2/D were detected in 2, 1 and 4 of our ESBL-positive isolates, respectively.
Conclusions: A high prevalence and diversity of ESBLs are detected in faecal samples of healthy humans, mainly of the CTX-M class. The gene blaCTX-M-14 has been found in structures related with insertion sequences or integrons.

P633 Characteristics of clinical extended-spectrum β-lactamase-producing isolates from companion animals in Finland

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Objective: Characterisation of ESBL-producing isolates of animal origin in Finland.
Methods: During a 4-year period 2004–2007, clinical isolates from animals yielded 16 *Escherichia coli*, seven *Enterobacter* sp., two *Acinetobacter* sp., one *Aeromonas* sp. and one *Klebsiella pneumoniae* non-susceptible to cefuroxime or cefotaxime. Bacterial identification was done with conventional biochemical methods and susceptibility testing with microdilution or disk diffusion method. Detection of TEM, SHV, CTX-M, and OXA genes, was performed by PCR and pyrosequencing. Pulsed Field Gel Electrophoresis (PFGE) was used for studying the clonal relationship of the isolates.
Results: Isolates were derived from twelve dogs, nine horses and five cats. For one isolate the animal species was not mentioned. Seventeen isolates were from urine, seven from wound discharge, two from vaginal discharge and one from faeces. Nine isolates carried an ESBL-gene. A CTX-M-1 gene was present in five isolates (4 *E. coli*, 1 *Aeromonas* spp.), while one isolate carried a CTX-M-9 gene (*E. coli*). Two isolates had an SHV-type gene (1 *K. pneumoniae*, 1 *Enterobacter* sp.), and one *Acinetobacter* sp an OXA-51 gene. The PFGE-profile was identical for two ESBL *E. coli* isolates from two different animals, although no regional or temporal relationship was apparent. ESBL genes were not detected in 18 isolates, but non-susceptibilty to 3rd generation cephalosporins suggests the presence of other mechanisms. Some of these isolates were resistant to cefoxitin which may indicate presence of AmpC.
Conclusion: Since isolates resistant to 3rd generation cephalosporins have emerged in companion animals, clinical laboratories should routinely screen bacterial isolates for resistance to these agents. Broad spectrum cephalosporins should be used judiciously in veterinary medicine.

P634 Prevailing occurrence of SHV-12-producing *E. coli* isolates in retail raw meat samples in southern Spain

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Introduction: Potential transfer of antimicrobial resistance from enteric zoonotic bacteria of food animals to humans is a cause of concern. Monitoring resistance is essential especially for important human therapeutic antibiotics as narrow spectrum cephalosporins. Currently, sick and healthy food animals are increasingly recognised as reservoir of extended spectrum β-lactamases-(ESBLs)-producing *E. coli*. Previous reports have focused on faecal samples from farm animals and abattoirs, but few data are available from commercial food supply.
Objective: a study was conducted to examine ESBL-producing *E. coli* (ESBLEC) in raw meat samples from Seville and to characterise their antibiotic resistance.
Material and Methods: A total of 94 prepackaged raw meat samples, including chicken, turkey, beef and pork, were purchased from retail stores (large chain, wholesale and deli grocery) in Seville from September through December 2006. After an overnight incubation in broth medium, homogenised were subcultured on McConkey agar supplemented with 1 mg/l cefotaxime or 1 mg/l ceftazidime. Lactose-positive colonies were identified by standard biochemical tests. Screening of ESBL production was done using the double disk diffusion method. ESBL characterisation was carried out by PCR assays with group-specific primers and sequencing. Strain relationship was studied by REP-PCR.
Results: Twenty five samples (27%) yielded 51 unrelated ESBLEC strains. ESBLEC was detected in chicken (13/15, 87%), turkey (7/12, 58%) and beef (1/12, 8%) and pork (4/55) (Chi^2, $p<0.001$). Twelve (37%) samples from the deli grocery were positives, 9 (26%) from the large chain and 4 (14%) from the wholesale store (Chi^2, $p=0.127$). Forty three (84%) strains produced SHV-12 and they came mainly from poultry (93%).
Conclusions: In our study a very high ESBLEC contamination was exhibited in raw poultry for human consumption. In contrast with previous studies, where CTX-M both M-1 and M-9 groups were the main enzymes associated with animals and food samples, the ESBL type predominantly detected in our area was SHV-12.

P635 Multidrug-resistant *Salmonella enterica* serovar Virchow encoding blaCTX-M3 located on an IncN plasmid isolated from Turkish food of avian origin

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Objectives: Characterisation of plasmid mediated extended spectrum cephalosporins resistance by a blaCTX-M3 in S. Virchow isolated from Turkish Food of avian origin.
Methods: 76 chicken meat samples were collected from different markets in Ankara, Turkey (2005–2006). All isolates were tested for their susceptibility to 17 antimicrobial agents, and ampicillin resistant

isolates were tested for an extra panel of 11 β-lactams. One S. Virchow PT26 (DMC19) isolate showed a resistance phenotype typical for ESBLs. The presence of blaCTX-M3 was detected by PCR/sequencing. The complete resistance repertoire (resistance genes, class 1 integron and mutations in the quinolone resistance determining regions) of this strain was characterised by molecular methods. The strain was typed by XbaI-PFGE and plasmid profile analysis. Location of ESBLs was determined by hybridisation assay. The plasmid was analysed by RFLP. The incompatibility group of the plasmid was investigated according to replicon typing by PCR. Conjugative properties were examined by bacterial matings.
Results: The S. Virchow isolate had a multi-drug resistance pattern of [AMP–EFT–CTX-CRO-CPD-ATM]–STR–SUL–TMP–SXT-NAL. Together with blaCTX-M3, the strain also carried blaTEM-1-strA/B-sul2-dfrA14 genes. A gyrATyr-87 mutation was responsible for the NAL resistance. No qnrA, B or S genes were detected. The strain did not possess any class 1 integron. The strain showed a common PFGE pattern like other S. Virchow isolates. It had only one plasmid of about 45 kb, named pBD2006. The blaCTX-M3 gene was located on this plasmid. After several intents the pBD2006 could not be transferred by conjugation. The plasmid belongs to the IncN incompatibility group.
Conclusions: blaCTX-M3 genes located in very big plasmids (300 kb) are wide spread among humans isolates in Mediterranean Regions. In the present study, the gene, located on a smaller plasmid, could be associated to food products of avian origin.

Molecular bacterial epidemiology

P636 Molecular identification and clonal dissemination of *Haemophilus haemolyticus* in acute pharyngotonsillitis

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Objectives: Bacterial infection plays an important role in the course and pathogenesis of acute pharyngotonsillitis. Although nontypeable *Haemophilus influenzae* (*H. influenzae*) is a human respiratory pathogen, *Haemophilus haemolyticus* (*H. haemolyticus*), the closest relative of *H. influenzae*, is considered to be a pharyngeal commensal. This study was designed to identify *H. haemolyticus* separate from *H. influenzae* by polymerase chain reaction (PCR). Clonal diversity of this pathogen was also evaluated by pulsed field gel electrophoresis (PFGE) in this study.
Materials and Methods: Clinical isolates from acute pharyngotonsillitis were studied to distinguish *H. influenzae* (38 strains) from *H. haemolyticus* (28 strains). *H. influenzae* and non-haemolytic *H. haemolyticus* were originally defined by typical colony morphology on chocolate agar, X and V growth factor dependency, and no reaction in the porphyrin test. Hemolysis was assessed as zones of beta-hemolysis surrounding individual colonies grown on horse blood agar to differentiate hemolytic *H. haemolyticus*. PCR primers were designed to amplify 16S rDNA, 7F3 epitope of outer membrane protein P6 and IgA protease gene (iga) to distinguish *H. haemolyticus* from *H. influenzae*. Clonal diversity of H. hemolyticus was also evaluated by PFGE.
Results: *H. haemolyticus* was distinguished from *H. influenzae* by three independent PCR sets. All strains of *H. influenzae* expressed the 7F3 epitope but lacked the 7F3 epitope in *H. haemolyticus*. The iga gene was also identified in only *H. influenzae*. PCR for 16S rDNA also could distinguish *H. influenzae* from *H. haemolyticus*. There was no clone identified in H. hemolyticus while nontypeable *H. influenzae* showed three clones.
Conclusion: *H. haemolyticus* is considered to be a pharyngeal commensal. It is important to distinguish the microorganism from causative pathogens. However, the standard methods can not reliably distinguish *H. haemolyticus* from *H. influenzae*. The recognition that some strains of apparent *H. influenzae* are *H. haemolyticus* is essential to understand association of true *H. influenzae* with respiratory tract infections.

P637 CagA variants in Iranian *Helicobacter pylori* isolates recovered from patients with different gastroduodenal diseases

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Objectives: The biological activity of CagA protein is related to the variable region of CagA containing variable numbers of EPIYA motifs. Binding of phosphorylated CagA to SHP-2 occurs with varying affinity. CagA is subdivided to the East Asian and Western subtypes based on its binding to SHP-2. The East Asian-type CagA binds to SHP-2 more strongly than the Western-type CagA. Due to the high prevalence of cagA-positive *Helicobacter pylori* (Hp) strains in East-Asian countries as demonstrated in our country, assessment of the presence or absence of cagA alone is not very informative. Consequently, CagA variants typing in term of the subtype and number of EPIYA motifs may be important in determination of the binding affinity of CagA to SHP-2 and the clinical relevance of cagA. The aim of this study was to characterise the CagA variants of Iranian Hp strains.
Methods: Genomic DNA was isolated from 142 Hp strain isolated from infected patients, followed by PCR amplification of the variable region of the cagA gene using cag2 and cag4 primers. Purified PCR products were sequenced. The sequence of each resulting subtype was deposited in the GenBank.
Results: One hundred and twenty four (87.3%) of examined isolates were cagA positive. Six CagA variants differing in length of PCR products were identified. Two to six EPIYA motifs were detected among the sequenced strains. The most prevalent (59.7%) PCR amplicon was 550bp designated as A-B-C type. All of the sequenced CagA variable region were identified as the "Western" type. Although there was no statistical association between any of the CagA subtypes and gastroduodenal disease, a specific CagA subtype yielded longer PCR product (750 bp), designated as A-B-B-B-C-C and was found exclusively in GC patients.
Conclusion: Evaluation of the variations in the cagA variable region seems as an effective method of screening for highly virulent cagA-positive strains. The primary finding of our study showed that Iranian stains have a lower binding affinity to SHP-2 than other East Asian strains. It is not unlikely that circulating of A-B-C type as the most prevalent type in Iranian dyspeptic patients and lower binding affinity of our strains to SHP-2 result in lower frequency of gastric cancer and peptic ulcer diseases in our population compared to other East Asian countries.

P638 cagA/vacA genotyping of *Helicobacter pylori* strains isolated from Cuban and Venezuelan patients

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Among the different genetic determinants involved in *H. pylori* virulence we find the citotoxin associated gene (cagA) and the vacuolating citotoxin gene (vacA). Different studies have shown the importance of the presence of these genes in the strain affecting a patient and the development of a gastric disease.
Objective: The aim of the study was to determine the presence of different *H. pylori* cagA/vacA genotypes among patients with gastritis in Cuba and Venezuela.
Methods: Biopsies were taken from 61 patients from Venezuela and from 71 patients from Cuba. Biopsies were cultured on selective media. DNA from the biopsies was obtained using a proteinase K protocol. A region of 349 pb of the cagA gene was amplified by PCR using the primers F1/B1, VA1-F/VA1-R set of primers to amplify the 259 bp (s1) or the 286 bp (s2) regions of the conserved portion of the vacA gene and VAG-R/VAG-F set of primers to amplify the 567bp (m1) or the 642bp (m2) regions of the conserved portion of the vacA gene. All the amplified regions were resolved on a 1% agarose gel electrophoresis at 80 volts for approximately 2 hours.

Results: The 349pb region of the cagA gene was amplified in 34 of the 61 Venezuelan patients (56%) and in 20 of the 71 Cuban patients (28%). We found a significant difference (p = 0.0015) between the presence of the cagA gene in both countries. When we analysed the prevalence of cagA related to the allelic variants we found a significant difference between both countries: in Venezuela we observed a significant higher percentage of the genotype cagA positive/m1s1, in comparison with Cuba (p = 0.0025). The percentage of cagA negative/m1s1 strains in Cuba was significantly higher than in Venezuela (p = 0.04). Out of the 71 patients from Cuba we found that in 8 of them there was co infection with a least two different strains since we detected both m1 and m2 alleles on them.

Conclusion: We found significant differences between the genotypes of the strains from both countries. Since the cagA and vacA genes are some of the virulence factors of *H. pylori* related to its pathogenesis and the severity of the gastric disease, these results are of great importance to the regional health systems of each country for the control, tracking and prevention of gastric cancer.

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P639 Qualitative and quantitative composition of gut microbiota from cystic fibrosis patients

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Objective: To analysed the gut microbiota of cystic fibrosis (CF) patients by classical culture-based methods and also by non-culture molecular approaches.

Methods: Faecal samples from 18 CF-patients and also from 20 healthy volunteers (HV) were seeded in selective agar plates including: M-*Enterococcus*, manitol-salt, MacConkey, MRS, and ESBL. An additional blood-Columbia agar plate was including in order to estimate the aerobic bacterial concentration in faeces, and also the organims not recovered on selective plates. Five different colonies per morphology was subculture in the corresponding plates, and their clonal relationship was assessed by PFGE. Antimicrobial susceptibility was tested in the different PFGE pattern clones by agar dilution method. Total DNA was obtained from the faeces (QuiAmp minikit, Quiagen) and quantitative-PCR experiments were conducted in order to know the bacterial density for the *Bacteroides–Prevotella–Porphyromonas*, *Fusobacterium*, and Lactic Acid Bacteria (LAB) groups. PCR-DGGE was performed with universal primers sets (V2-V3 region of 16S) and also with the described bacterial groups.

Results: Considering the blood-Columbia agar plate, lower bacterial charge was found in the CF faecal samples than in the HV. Curiously, Gram-negative bacteria were not detected in CF samples, neither in the selective mediums (MacConkey and ESBL) nor in the blood-Columbia agar. Enterococcal and staphylococcal clones were detected only in 10 and 9 CF patients, respectively, whereas for HV these genera were represented in all subjects. Several LAB genera as *Lactococcus*, *Lactobacillus*, and Pediococcus were detected in almost 50% of the CF samples. Multirresistance to four or more antibiotic families were corroborated in all CF isolates. Quantitative-PCR results showed statistical differences among the density proportions of the bacterial groups in the CF-patients versus the HV: *Bacteroides–Prevotella–Porphyromonas* (6:1), *Fusobacterium* (1.8:1), and LAB (2:1) groups. DGGE-experiments showed particular bands associated only to CF-patients.

Conclusion: Qualitative and Quantitative significant differences were detected in the CF gut microbiota when comparing with that of HV, being relevant the higher amounts of anaerobes in the CF faces and the absence of Gram-negative cultivable organism. These differences can be related with antimicrobial selective pressure and particular gut mucosal conditions in CF patients.

P640 Serotype distributions and molecular characteristics of *Vibrio parahaemolyticus* isolates in Malaysia

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Objectives: To determine the serotype distributions of *Vibrio parahaemolyticus* isolates in Malaysian clinical isolates and to identify the genotypic characteristics of the strains.

Methods: A collection of fourteen *V. parahaemolyticus* clinical isolates from various hospitals and National Public Health Laboratory in Malaysia from year 1997 to 2000 and 2005 to 2007 were serotyped using commercial O and K antisera. The isolates were subjected to PCR analysis to detect species-specific toxR gene of *V. parahaemolyticus*, tdh and trh toxin genes, presence of orf8 gene and confirmation of pandemic strain by group-specific PCR (GS-PCR).

Results: From the fourteen clinical isolates, eight were serotyped as O3:K6 making it the dominant serotype (57.14%), followed by O4:K68 representing three isolates (21.43%) and one strain each representing serotype O1:KUT, O3:K5 and O4:K8 respectively. All of these isolates were positive for the toxR gene and were confirmed as *V. parahaemolyticus*. All the isolates were also positive for the tdh toxin gene except for one strain of serotype O1:KUT. None of the isolates were positive for the trh gene. Only those of serotype O3:K6 and O4:K68 possessed all genotypic characteristics of the pandemic strain, being positive for the tdh gene and negative for trh gene, positive for the orf8 structural gene and were also GS-PCR positive. However, the three other serotypes were negative for orf8 gene and were GS-PCR negative.

Conclusion: This study showed that pandemic serotypes are present in Malaysia and are represented by serotype O3:K6 and O4:K68. In this limited number of isolates, O3:K6 and O4:K68 were shown to be the dominant serotypes. These particular strains possessed all structural genes of the pandemic strain which were further confirmed by GS-PCR method.

P641 Faecal prevalence and strain diversity of *Listeria monocytogenes* in healthy cattle, sheep and swine herds in northern Spain

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Objectives: Although the incidence of human listeriosis is low compared to salmonellosis or campylobacteriosis (0.88 cases per 100,000 inhabitants per annum in the last 5 years in the Basque Country), *Listeria monocytogenes* is among the most important foodborne bacterial pathogens due to the high mortality rate (20–30%) and severity of the disease. *L. monocytogenes* is a ubiquitous organism that inhabits the intestinal tract of various livestock species that act as major reservoirs and source of human infection via faecal contamination of food products. The aim of this study was to determine the prevalence and strain diversity of *L. monocytogenes* in healthy ruminants and swine farms.

Methods: Faecal samples from 30 animals per farm were collected from 343 farms (120 ovine, 124 beef cattle, 82 dairy cattle and 17 porcine) and screened in pools by an automated enzyme-linked fluorescent immunoassay (VIDAS® LMO2, bioMérieux). Positive samples were subcultured onto the selective and differential agar ALOA and biochemically confirmed. Within-herd prevalence of *L. monocytogenes* was investigated by individually analysing 50 animals from a selection of pool-positive herds. Isolates were characterised by serotyping and ApaI PFGE analysis.

Results: *L. monocytogenes* was isolated from 46.3% of dairy cattle, 30.6% beef cattle and 14.2% ovine farms, but not from swine. Within-herd prevalences established by individual testing of 197 sheep (4 herds) and 221 cattle (5 herds was 1.5% (0.0–4.1%) and 21.3% (5.1–72.3%), respectively. Serotyping of 114 isolates (20 ovine and 94 bovine) identified complex 4b the most prevalent (81.7%), followed by 1/2a (14.0%), 1/2b (2.1%) and 4c and 4e (1.1% each). Distribution of serotypes varied slightly among the different host species. PFGE analysis performed on 67 isolates indicated that the *L. monocytogenes* population

in Basque farms is genetically highly diverse and identified the presence of different strains within each farm.

Conclusion: Prevalence values observed in ruminants indicate that good management practices and control strategies at the production level are crucial to reduce *L. monocytogenes* contamination in the live animal at the primary production level before entry to the slaughterhouse to reduce risk of human infection.

P642 Phylogenetic grouping of *Escherichia coli* isolates from patients' stool samples with diarrhoea

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Objectives: *Escherichia coli* is a normal inhabitant of the gut flora but is also frequently isolated in cases of infections. The population structure of extraintestinal strains has been studied during the last decade through the assignment of *E. coli* isolates to one of the four main phylogenetics groups (A, B1, B2 and D). Since less data are available for *E. coli* causing intestinal infections, the aim of our study was to assess the implication of the genetic background of selected intestinal *E. coli* isolates.

Methods: 66 *E. coli* isolates from patients' stool samples with diarrhoea of unknown origin, were assigned to one of the phylogenetic groups using the method described by Clermont et al..Virulence genes vtx1, vtx2, eae, ipaH, eltA, and estA were detected by PCR using the "DEC Primer Mix" produced by Statens Serum Institut, Denmark. EHEC-hlyA was determined on enterohemolysin agar plates, Oxoid, England and by PCR using primer pair hlyen1/hlyen2 (5′-GGT GCA GCA GAA AAA GTT GTA G-3′/5′-TCT CGC CTG ATA GTG TTT GGT A-3′). The O antigens were determined by using antisera produced by the Institute of Immunology, Croatia and Sifin, Germany.

Results: 10, 17, 25 and 14 isolates were placed into the phylogenetic groups A, B1, B2 and D, respectively. Serological groups found within group A isolates were O5, O6, O15, O75 and O86. Only one of the two O157 isolates within this group carried an eae gene. In none of the other nine isolates vtx1, vtx2, eae, ipaH, eltA, or estA genes were detected. To our surprise all of the 12 determined O26 isolates were placed into group B1. All 12 isolates carried an eae gene, nine were EHEC-hlyA positive and two carried additional the vtx1 or vtx2 gene. The most prevalent O serological groups found within B2 phylogenetic group were O1, O2, O4, O6, O18 and O142. One of the isolates was O157 with a detected eae gene. 14 isolates were placed into group D. In all of the six determined O157 isolates the eae gene was detected. Additionally the O157 isolates carried either the EHEC-hlyA and/or the vtx2 gene. The eae and EHEC-hlyA genes were also found in the two O145 isolates from phylogenetic group D.

Conclusions: The majority of the O26 and O157 isolates associated either with eae, vtx1, vtx2, or EHEC-hlyA genes were placed into the B1 or D phylogenetic group. Thus, phylogenetic grouping could be partially used in screening projects for a rapid assessment of potentially pathogenic *E. coli* strains (EPEC and VTEC associated) in Slovenia.

P643 Ciprofloxacin resistance, virulence determinants and phylogenetic background of uropathogenic *Escherichia coli* isolates from general practice patients in Slovenia

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Objectives: Urinary tract infections are mainly caused by uropathogenic strains of *Escherichia coli*. Knowledge on virulence traits and phylogenetic background of extraintestinal (ExPEC) *E. coli* has increased tremendously over the last years. Results of several studies revealed, that human ExPEC strains mostly belong to phylogenetic group B2 followed by group D, and that quinolone-resistant ExPEC strains have less virulence factors (VFs) than quinolone susceptible strains. The aim of our study was to gain insight into the genetic difference between ciprofloxacin susceptible and resistant strains recovered from urinary tract infections of a random population of patients subjected to empirical antimicrobial treatment in general pratices in the Ljubljana region.

Methods: *E. coli* isolates recovered from patients with symptoms of urinary tract infections were collected and identified at the Institute of Public Health of the Republic of Slovenia. A random sample of 44 ciprofloxacin resistant and 53 ciprofloxacin susceptible isolates, as determined by the disk diffusion method according to the CLSI standards, were included in the study. All isolates were placed into phylogenetic group A, B1, B2 or D as described by Clermont et al.. The isolates were screened by PCR for 23 virulence genes, including adhesins, serum resistance genes, toxins, invasins, and genes involved in iron aquisition.

Results: Alltogether, 16.5%, 5.1%, 45.4% and 33% of the isolates were placed into phylogenetic group A, B1, B2 and D, respectively. Among the ciprofloxacin susceptible isolates the most prevalent group was B2 (56.6%), followed by group D (30.3%), whereas the resistant isolates were almost equally distributed within groups D, B2 and A (36.2%, 32% and 27.3%). Virulence genes were found in both resistance groups. However, there was an obvious difference in the distribution of kpsMTII, usp and iroN genes, being detected in 71.7%, 30%, 67.9%, and in 43.1%, 13.6% 38.6% of the ciprofloxacin susceptible and resistant isolates, respectively. The prevalence of virulence genes varied significantly between isolates from different phylogentic groups and also within each group if the isolates were divided into subgroups according to the ciprofloxacin resistance profile.

Conclusions: Since non-B2 ciprofloxacin resistant *E. coli* isolates with less VFs, are frequently isolated from general practice patients, they have to be considered as important uropathogens.

P644 M serotyping of group A beta haemolytic streptococci isolated from throat infection by emm-gene sequence analysis from Turkey

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Objective: Following group A beta haemolytic streptoccoci related throat infections, invasive diseases may occur due to several virulence factors such as M protein. The purpose of this study is to determine emm types of Turkish people suffering throat infections and investigate the relation between M-types, sex, age and type of diseases.

Methods: 114 *S. pyogenes* strains were isolated from throat cultures and identified with conventional microbiological methods (culture, serotyping, API Strep®). emm specific PCR products of these strains were obtained using emmP1, emmP2 primers recommended by Centers of Disease Control. emm types of the strains were sequenced by DNA cycle-sequencing analyse. Local and global alignments were performed by Clustal W v1.7 software and Blast server. Phylogenetic dendrogram was drawn. The correlation between "emm" types and sex of the patients have been evaluated by Chi-square test statistically. The distribution of age and "emm" types of the patients were analysed by Mann-Whitney U test.

Results: Of the 114 strains (85%), 97 strains' emm types were identified as 1, 3, 4, 6, 9, 11, 12, 14, 18, 22, 24, 29, 33, 43, 48, 75, 77, 78, 89, 90, 102, 112, 118. The other 17 types could not have been identified and accepted as nontypeable. The most frequently seen types were emm 12, 3, 1, 75, 29 respectively which were isolated from school age children under 10-years-old. The patients' age distribution was found variable but mostly accordant to emm types which were identified from invasive streptococcal infections. It was found that there's no statistically significant difference between the emm types and the sex of the patients. Besides, 15 of emm/M types which were determined in this study, including the most frequently seen types (emm 1, 3 and 12) were found that they were also in the contents of M types of 26 VV vaccine (suggested to be effective as 76.9%) developed for Group A streptococci (GAS)

Conclusion: This preliminary study is the first emm-gene sequence analysis presented from Turkey which determines the M types of GAS strains in our country. If the research can be generalised to all different regions we will get more exactly and comprehensive results about the M types of Turkish people.

P645 Molecular epidemiology and pathogenesis of pneumococcal cerebral abscesses in Scotland

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Objectives:
- To document the molecular epidemiology of pneumococcal cerebral abscesses in Scotland.
- To perform microarray DNA comparative genomic hybridisation (CGH) using isolates from the commonest multi locus sequence type (MLST) and serotype to document regions of diversity in their genomes.
- To assess in vitro expression of virulence-associated genes from these isolates.

Methods:
- The Scottish Meningococcal and Pneumococcal Reference Laboratory database of invasive pneumococcal cases was searched for cases of cerebral abscess where pneumococci had been grown from pus.
- DNA was extracted from overnight cultures at 37ºC using a phenol chloroform extraction method. RNA was extracted from cultures in brain heart infusion broth at 37ºC grown to an optical density at 600nm of 0.6 using an RNeasy Mini kit (Qiagen). Fluorochrome Cy^{TM}3 or Cy^{TM}5 dCTP (GE Healthcare) was incorporated into DNA or cDNA as part of a randomly primed polymerisation reaction. Competitive hybridisation against reference DNA or cDNA extracted from TIGR4 (a fully sequenced serotype 4 isolate) was performed on prehybridised glass slide microarrays constructed by robotic spotting of PCR amplicons. Slides were scanned (ScanArray®, Packard Biosciences) and data analysed using Bluefuse (BlueGnome) and Genespring GX 7.3.1 (Agilent Technologies) software.

Results:
- Of 8 identified cases, 5 were male and 5 died from their disease within 30 days of culture (overall case fatality 62%). The serotypes seen (n = 8) were serotype 3 (37%), 4 (24%), 10A (13%), 12F (13%) and 19F (13%). MLST data was available for 6 isolates – ST180 (50%), ST162 (16.7%), ST218 (16.7%), ST246 (16.7%).
- DNA CGH of two serotype 3 ST180 isolates suggested that gene deletions were present in several previously recognised regions of diversity.
- RNA expression microarray analysis identified several up-regulated, virulence associated genes. The functions of relatively few of these genes were known but included an iron ATP transporter – ATP binding protein (SP0242), hyaluronidase (SP0314) and a serine protease (SP2239).

Conclusions:
- Most cases of pneumococcal cerebral abscess in Scotland are not preventable using existing formulations of pneumococcal conjugate vaccine.
- A microarray approach to identify which genes are present and expressed by pneumococci causing this condition can identify virulence associated genes and their products which could be targeted by alternative prevention strategies or novel vaccines.

P646 A case of cutaneous listeriosis in a man

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Objectives: In humans, listeriosis caused by *Listeria monocytogenes* usually occurs in certain high-risk groups (neonates, pregnant women, immunocompromised adults) but rarely in persons without predisposing factors. Sepsis, meningitis and meningoencephalitis are common features of listeriosis in humans; rare cutaneous form of the disease is linked mainly to occupational hazards (veterinarians, laboratory workers).

A case of *L. monocytogenes* skin infection in a man is presented. A 54-year-old male veterinary practitioner developed pustular changes on the skin of arms and hands after assisting the delivery of stillborn calf. During the parturition one of his gloves tore and he continued with exposed hands. He suspected the bacterial infection to be the cause for stillbirth therefore he took a sample of placenta and sent it to the laboratory for bacteriological examination. Later, pustular changes developed on his skin; the swabs were taken for bacteriology and *L. monocytogenes* was isolated. The patient was treated successfully with amoxicillin and clavulanic acid.

Methods: Cultivation, identification, serotyping and genotyping of the strains from pustulae and placenta were performed. As a genotyping method, pulsed-field gel electrophoresis (PFGE) was used and performed following the CDC standardised PulseNet protocol for *L. monocytogenes* using restriction endonucleases AscI and ApaI. The genotyping results were analysed using BioNumerics software (Applied Maths).

Results: Pure cultures of small haemolytic colonies suspicious of *Listeria* spp. were grown after 24h incubation on sheep blood agar from all specimens. The colonies were identified as *L. monocytogenes* with biochemical kit API *Listeria* (BioMerieux). Serotyping resulted in serotype 4b in all isolates. The cleavage of the DNA with both AscI and ApaI exhibited identical PFGE restriction patterns of all strains suggesting the zoonotic transmission of the infection with *L. monocytogenes*.

Conclusion: To the best of our knowledge, this is the first case of cutaneous listeriosis where the evidence for zoonotic transmission of *L. monocytogenes* was supported by genotyping methods.

P647 Phylogenetic analysis of genus salmonella based on polyphasic and numerical taxonomy

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Objective: The genus *Salmonella* is a member of the family Enterobacteriaceae. There are over 2500 serotypes of this genus based on antigenic differences associated with gasteroenteritidis and enteric fever (typhoid) in human. The division of *Salmonella* into seven major groups has been confirmed by sequence analysis of house keeping, 16S rRNA and invasion genes.

In this study two method of phylogenetic relationship were used. Sequencing method of 16S rRNA and gyrB used to polyphasic taxonomy and RAPD-PCR were used to numerical taxonomy.

Method: Fourty and thirty six strain which isolated from clinical samples were used to sequence and RAPD-PCR analysis respectively. After serotyping their DNA were extracted by phenol chloroform method and PCR amplification and RAPD-PCR were done by universal, sequencing and random primers.

At last PCR products run on agarose gel 1.2% and staining with Ethidium Bromide.

Sequencing results analysed by Clustal X, DNA Star, BioEdit, MEGA4 and RAPD-PCR results was analysed by NTSYS 2 softwares.

Result: The similarity with 16S rRNA gene sequence99.7%, 23S rRNA 98.4–100%, gyrB 97.2–99.8% and amino acid sequence of gyrB 99.8–100% were obtained.

According to the results, 16S rRNA sequences showed poor separation at or below species level. 23S rRNA gene sequence data was better than 16S rRNA to analyse differentiation at the species, subspecies level. The gyrBgene (topisomerase type II) is useful to analyse closely related species at subspecies and serovar level. It was observed that gyrB separation is better than 23S rRNA sequences.

The results of RAPD-PCR were defferent. Based on the primer similarity patterns were different, but the average of them observed between 70–89%.

Conclusion: RAPD-PCR results showed that this method is useful to analyse similarity between several serotypes of *Salmonella*. It is not suitable to Identify unknown isolates according to the genetic pattern of RAPD-PCR.

In contrast of sequencing method this technique is cheap and easy, but it's resolution and sharpness less than sequencing method.

So sequencing method is the best for characterisation and identification of *Salmonella*'s strains for epidemiology and phylogenetic study. A large

number of groups within Bacteria can define and distinguished from each other by this method.

Carriage of MRSA and MSSA

P648 The carriage population of *Staphylococcus aureus* from Mali is composed of a combination of pandemic clones and the divergent genotype ST 152

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Objectives: To characterise a sample of *Staphylococcus aureus* isolates from asymptomatic carriage in Mali, Africa, and to compare the results to the known diversity of the carriage population in order to evaluate the role of microepidemic and pandemic clonal spread in shaping *S. aureus* population structure.

Methods: Nasal swab was performed in 448 patients admitted for emergency surgery to a single hospital point G in Bamako. *S. aureus* isolates were characterised by multilocus sequence typing and the results compared against the entire MLST data set and a previous study of carriage isolates from the UK. We used a combination of eBURST and phylogenetic approaches to analyse the data.

Results: Prevalence of nasal *S. aureus* carriage was 19.6% (n = 88). Carriers differed from non carriers for gender (51% vs 35%) and for mean age (41 vs 51.6) ($p < 0.05$) but not for chronic health status and previous hospitalisation. Only one isolate was meticillin resistant. Of the 87 meticillin susceptible isolate, 97% were resistant to penicillin, 6% to erythromycin and 74% to tetracycline. The 88 isolates characterised by MLST were found to correspond to 20 STs. Eighteen of these STs were present in the MLST database and two STs were new. No new alleles were detected. Seven of the 20 STs (ST5, ST8, ST15, ST30, ST88, ST152, ST291) were found in at least 4 strains, 4 of the STs (ST1, ST101, ST120, ST852) were found in 2 or 3 strains, and 9 of the STs corresponded to only a single isolate. We note the presence of many of the commonly recorded *S. aureus* clonal complexes in the Malian carriage population (CC15 (28%), CC8 (8%), CC5 (7%), CC30 (4.5%), CC88 (4.5%), CC45 (3%), CC121 (2%)) but also a high frequency (~24%) of the previously rare genotype ST 152.

Conclusion: Our results confirm the pandemic spread of many *S. aureus* clonal complexes, an observation which challenges purely neutral hypotheses regarding their origin and maintenance. In contrast, the high prevalence of ST 152 demonstrates that microepidemic transmission may also be important, and that much of the diversity of *S. aureus* population remains to be uncovered.

P649 Frequency, molecular typing and association with clinical disease of *Staphylococcus aureus* Panton-Valentine leucocidin positive isolates in a tertiary hospital

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Objectives: To evaluate the frequency, the molecular typing and the association with clinical disease of *S. aureus* carrying Panton-Valentine leucocidin (PVL) from hospitalised and outpatients during 22 months (1/2006–11/2007)

Methods: During the study period 405 *S. aureus* strains were isolated from soft tissue abscesses, purulent skin infections and nasal swabs obtained from hospitalised and outpatients. *S. aureus* strains were identified by conventional methods and automated system VITEK 2 (bioMerieux). The antibiotic susceptibility testing was performed by disk diffusion agar method and the determination of MICs by VITEK 2. PVL genes (lukF-PV, lukS-PV) and SCCmec types were determined by PCR, while the genotypes of the isolates were investigated by pulsed field gel electrophoresis (PFGE)

Results: A total of 405 *S. aureus* strains were isolated from different patients (312 from hospitalised and 93 from outpatients). MRSA strains were 228 out of 405 (56.3%). Especially 61.5% (192/312) MRSA strains were recovered from different wards of the hospital and 39.8% (37/93) from outpatients. PVL+ strains represented 9.6% (39/405) of the isolated *S. aureus* strains (38 community acquired (CA) and only one hospital acquired) while PVL+ strains represented 16.7% (38/228) of the isolated MRSA. All of the CA MRSA strains were PVL+ (37/37, 100%) and one MRSA PVL+ strain was isolated from nosocomial infection (1/192, 0.5%). One MSSA PVL+ was isolated from an outpatient. The origin of PVL+ strains was: abscesses (25), nasal carriage (7), furunculosis (3), cerebrospinal fluid (CSF) (1), cellulites (1), pyomyocitis (1), peritoneal catheter (1). All of the MRSA and MSSA PVL+ strains were SCCmec type IV by PCR, while 13 PVL+ strains were also investigated by PFGE and showed only one clone ST80. All the MRSA PVL+ isolates were resistant to oxacillin (OXA), kanamycin (KA), tetracycline (TE) and intermediate susceptible to fucidic acid (FA).

Conclusions:

1. In our hospital the prevalence of *S. aureus* PVL+ strains was approximately 10%, while the prevalence of the MRSA PVL+ strains was approximately 17%
2. All CA MRSA and one CA MSSA strain were PVL+ SCCmec type IV and 13 of them were genotyped as ST80
3. One MRSA PVL+ strain was isolated from CSF of a hospitalised patient with clinical signs of meningitis
4. The MRSA PVL+ strains had the same unique resistance pattern to antibiotics (resistance to KA, OXA, TE and intermediate resistance to FA)

P650 PVL(+) MRSA causing infections in hospitalised patients

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Objectives: Concerns for Panton-Valentine leukocidin (PVL) producing community-associated (CA) MRSA strains overtaking healthcare-associated (HA) infections and the potential for severe infections in association with PVL expression level have led us to determine 1) molecular epidemiology, 2) disease spectrum, and 3) correlation of disease severity with PVL gene expression level in hospitalised patients infected with PVL(+) MRSA strains.

Methods: 186 MRSA isolates obtained from patients during 7/05–6/07 were saved for molecular characterisation while medical charts of patients were reviewed for relevant demographic, laboratory, and clinical data. We screened all isolates for the presence of lukS-F gene by PVL Evigene® test (AdvanDx, Woburn, MA: a qualitative nucleic acid hybridisation assay for research use only). Using published protocols, PCR typing for SCCmec types (I-IV), USA300;ST8 and PVL transcripts by reverse transcriptase-PCR using SYBR green with primer specific for lukF gene were performed on a subset of strains. MRSA NRS384 (PVL(+) USA300;ST8, SCCmec IVa) and NRS22 (PVL(–), USA600;ST45, SCCmec II) strains served as positive and negative controls respectively.

Results: PVL(+) strains caused 45% (83/186) of MRSA infections in hospitalised adults; 54% were HA per clinical criteria. 96% of PVL(+) strains (52/54) belong to USA300 and 85% (46/54) carried SCCmec type IV cassette. CA-infections were 92% cellulitis +/– abscess plus 1 necrotising fasciitis. HA-infections were more likely to be invasive (pneumonia, bacteraemia) than CA (53% vs 5%, $p < 0.05$), with 60% from nursing home residents. Patients with HA compared to CA infections were older (mean age: 68 vs 48y, $p < 0.0001$) with higher APACHE II score (12.3 vs 3.4, $p < 0.0001$). Of the PVL(+) isolates tested (n = 61), pvl transcript levels ranged from 0 to 8.9 fold higher than control strain. When grouped based on level of pvl gene expression, strains with 2-fold higher mRNA levels vs 0–2 fold relative to control strain were more frequently CA (57% vs 43%) and less likely to cause invasive infections (43% vs 55%) such as pneumonia (7% vs 32%).

Conclusion: Over half of the USA300, SCCmec IV, PVL(+) strains caused healthcare rather than community-associated MRSA infections in adults, with the predominant place of acquisition in the nursing home.

PVL expression levels vary widely among clinical isolates; high PVL expression levels do not predict invasive disease.

P651 Gastro-intestinal carriage of MRSA in patients with community-acquired diarrhoea

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Objectives: There is increasing concern about cases of infection with meticillin resistant *Staphylococcus aureus* (MRSA) in the community, but comparatively little work has been done on reservoirs for MRSA outside the hospital. Since *S. aureus* has the potential to colonise the gut and may be enterotoxigenic, we sought to determine the prevalence of MRSA in diarrhoeal samples originating from the community.
Methods: During a year-long survey, faecal samples from non-hospitalised patients submitted for standard investigation of diarrhoeal symptoms were also screened for *S. aureus* by the diagnostic laboratory. Samples were cultured on mannitol salt agar at 37ºC in aerobic conditions for 48 hours. *S. aureus* isolates were identified by latex agglutination and DNAse activity, and MRSA were confirmed by resistance to cefoxitin.
Results: Of 2624 faecal samples investigated, 405 (15.4%) tested positive for the presence of *S. aureus*. Of these, 384 (14.6% of all samples) yielded meticillin sensitive *S. aureus* (MSSA) and just 21 (0.8%) yielded MRSA. The latter originated from patients aged 3 months to 95 years and 15 (71%) of these had no previous history of colonisation or infection with MRSA. However, the majority, (16/21 or 76%) of the MRSA isolates came from patients aged over 75 years. The overall carriage rate of MRSA in stool samples from non-hospitalised individuals aged over 75 years, with diarrhoea was 11% (16 of 144 specimens). This compares to an MRSA gastrointestinal prevalence of 7.6% amongst elderly patients with nosocomial diarrhoea in our hospital over the same time period.
Conclusion: MRSA colonisation is a risk factor for subsequent infection. This study has demonstrated that the gastrointestinal tract can be a significant reservoir of MRSA in the over 75s living in the community, and it is possible that strains are being disseminated via diarrhoeal episodes. This has consequences for control of MRSA in the community, where emphasis is often placed on controlling just nasal and skin colonisation. Further studies of faecal MRSA are warranted, including their enterotoxin status and risk factors for gastrointestinal colonisation.

P652 Prevalence of and risk factors for colonisation with meticillin-resistant *Staphylococcus aureus* at the time of hospital admission

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Objective: To determine the prevalence of MRSA colonisation among patients presenting for hospital admission, its correlation with community-acquired MRSA (CA-MRSA) prevalence and to identify risk factors for MRSA colonisation
Methods: In a tertiary teaching hospital, surveillance cultures from the nares, axilla and inguinal areas were performed at the time of admission for all patients except those admitted in the oncology and hematology unit. Demographic information and possible risk factors for colonisation were also recorded.
Results: Swab samples were collected from 937 patients presenting for admission in a year. Patients lived in urban areas in 87%, had a mean age of 57years (median 63) and a female to male ratio of 1.1:1. *Staphylococcus aureus* was isolated from 142 (15.1%) patients and MRSA from 30 (3.2%) of the patients (20% of colonising isolates). Detection of colonisation was 88.7% in the nares, 40.8% in the axilla and 51.4% in the inguinal area. In 3 (2.7.%) and 7 (4.9%) colonisation was only evident in the axilla and inguinal area respectively.
All colonising MRSA isolates exhibited in their sensitivity tests the pattern expected for CA-MRSA with the majority of them sensitive to cotrimoxazole, clindamycin, minocycline, rifampin and quinolones. Interestingly tetracycline and fucidic acid were inactive in the majority of strains. In the multivariate analysis comparing MRSA-colonised to MSSA-colonised patients, independent predictors of MRSA colonisation were: age <50 ($p=0.017$), the presence of an indwelling urinary catheter ($p=0.036$), the absence of hospitalisation the preceding year ($p=0.02$) and the presence of skin disease ($p=0.009$).
Conclusion: Colonisation with MRSA of patients admitted to the hospital is low and probably reflects the prevalence of colonisation with CA-MRSA in the community. Risk factors for MRSA acquisition do not correlate with previous contact with healthcare facilities but with younger age, skin diseases (affecting skin integrity) and debilitating conditions (as expressed by the presence of an indwelling urinary catheter). Larger sample of data may be required to further explore risk factors associated with MRSA colonisation in the community.

P653 Identification of the transmission vectors of *Staphylococcus aureus* circulating in a neonatal intensive care unit

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Objectives: The neonatal intensive care unit (NICU) of Hospital Fernando Fonseca, Lisbon, Portugal assists an average of 450 newborns/year and employs 100 healthcare workers (HCW).
Previous reports revealed that in 2002, 22% of the infections at the NICU were due to staphylococci. Identification of the reservoirs and routes of transmission of these microorganisms is a matter of major concern.
Methods: Between July 2005 and June 2007, 10 infection episodes occurred at the NICU. *Staphylococcus aureus* isolates were recovered during each episode from the infected babies, from nasal swabs of parents and HCW that had contact with the babies, and from the environment. The isolates were characterised by PFGE, spa typing and multilocus sequence typing. Virulence determinants were detected by multiplex PCR.
Results: All but one of the 10 infection episodes (chronologically designated I to X) included a single infected baby. None of the 30 *S. aureus* strains recovered was resistant to meticillin. Molecular characterisation distributed the 30 MSSA isolates into eight PFGE patterns (A to H). The majority of the isolates ($n=20$, 67%) belonged to two major clones: A (spa type t1228 and ST5, $n=11$) and B (t012 and ST30, $n=9$). Although none of the isolates was PVL positive, all except PFGE pattern B strains were positive for LukE-LukD and gama-hemolisin variant determinants.
In two episodes, isolates belonging to the same PFGE type, but a different subtype, were recovered from both a baby and a HCW (A1, A2 in episode II and B4, B5 in episode X, respectively). No identical isolates from babies and parents and/or environment were found in the same episode. Nevertheless, a HCW isolate from episode II showed the same PFGE subtype (B1) of the babies' isolates in episodes I and VII and a similar type with isolates from babies in episodes VI (B3) and X (B4). However, no direct contact was reported between the four infected babies and this HCW. In addition, in episode VIII the baby was infected with the same strain (PFGE A3) recovered from a HCW and the environment in two anterior episodes (III, VI).
Conclusions: Our data suggests that a *S. aureus* transmission route could be traced between a HCW and the babies in two infection episodes (II and X). Highly related isolates belonging to two major clones were periodically recovered during epidemiologically unrelated infection episodes, which might indicate that these strains are endemic at the NICU.

P654 **The search and destroy strategy does prevent spread and long-term carriage of MRSA; results from household screening following a ST22 (E-MRSA 15) outbreak**

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Objectives: From November 1, 2002 to December 31, 2005 440 persons were infected/colonised with MRSA ST22 during a hospital outbreak in Vejle County, Denmark.
This study was made to define the carriage rate, treatment outcome and spread of MRSA in households of previous ST22 infected/colonised patients and hospital staff.
Methods: The household visits were carried out from December 2006 to March 2007 and included samplings from all household members and pets as well as from the environment. Household members were sampled from nose, tonsils, urine and skin lesions; pets were sampled from nose, anus and skin lesions; and the environment from telephone, favourite chair, toilet flush handle, TV remote control and skirting board by the bed. Swabs (Regular flocked swabs, Copan, Italy) from human and animal samples were incubated in TSB (SSI diagnostics, Denmark). PCR positive broths were sub-cultured on blood agar (SSI) for final ID and susceptibility testing.
Urine samples were cultured on Chromogenic MRSA agar (Oxoid, UK). Environmental sampling was performed with Biotrace Dipslides (Biotrace, UK). Isolates suspected to be MRSA were confirmed and typed using mecA, SCCmec and spa PCR.
Following household visits, MRSA positive households received cleaning instructions, MRSA eradication treatment and follow up according to local MRSA-guidelines.
Results: Samples were obtained from 102 out of 251 households; 88 (45%) patients, 15 (27%) hospital staff, plus 45 relatives of patients, 22 relatives of hospital staff and 31 pets. 20 MRSA positive persons were identified; 16 patients (15 ST22, 1 ST5) and 4 patient relatives (2 ST22, 2 ST5). All households of hospital staff were negative. The 31 pets from 23 households were also negative. MRSA was found in tonsils (80%), nose (74%), and in urine (16%); from catheterised patients only. MRSA was found in the environment of 4 presently positive, long-term MRSA ST22 carriers. 88% of the positive patients had previously received MRSA treatment. After re-treatment 4 patients were still positive: 2 with catheter, 1 with fistulas and 1 with alcohol abuse. The mean follow-up time was 11 months.
Conclusion: Follow-up on 103 previously MRSA ST22 positive patients/hospital staff showed that 20% of patients were positive; following re-treatment 95% were MRSA negative. The environment was a possible reservoir for re-colonisation in households of long-term and present carriers. No pets were found positive for MRSA.

P655 **Meticillin-resistant *Staphylococcus aureus* in young dogs and their owners**

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Objectives: An outbreak of neonatal mortality in dogs by a Meticillin-resistant *Staphylococcus aureus* (MRSA) is reported and the possible role of the owners as reservoirs of the infection is evaluated.
Case presentation and results: A female boxer at the first pregnancy gave birth to 12 pups without post-partum complications. As the dam developed agalactia, the pups were fed with artificial milk. The entire litter died within 10 days with signs of severe septicaemia. The bitch was living with a male boxer and both the dogs tested negative by seroagglutination for Brucella canis. One pup was sent to the laboratory for necropsy. Multifocal haemorrhagic lesions in various organs were observed. Tissue samples from the organs were subjected to investigations for viral and bacterial pathogens. By PCR, the samples tested negative to Canine Herpesvirus and Canine Parvovirus type 1. By bacteriological analysis, a strain of *S. aureus* was isolated in purity from heart, liver, spleen and kidney of the pup. The strain was found to be Meticillin resistant by a PCR assay specific for the mecA gene.
In order to investigate the source of the infection by MRSA, bacteriological analysis was extended to the dogs and persons living in the household. Swabs from several parts of the body were taken from the parents of the pups, from the 2 owners (a man and his wife), and from a relative, a woman who occasionally looked after the animals. The man and this woman worked as medical attendants of the same hospital. From all the samples MRSA was isolated, for a total of 17 strains.
The strains exhibited the same pattern of antimicrobial susceptibility, being resistant to more than 3 classes of non-β-lactams. All the strains possessed the staphylococcal chromosome cassette mec (SCCmec) II and lacked the Panton valentine leucocidin (PVL) genes. In addition, they displayed the same pulsotype, thus suggesting a clonal origin.
Conclusion: From dogs and humans living in the same household a single MRSA clone was isolated displaying typical pattern of HA-MRSA (multidrug resistant, SCCmec II type and PVL negative). Since two of the persons were medical attendants in a hospital, and the dogs had no contact with other animals or persons, we hypothesise that a MRSA clone spread from the hospital and got adapted to the household, causing a fatal infection in the pups.

P656 **Prevalence of meticillin-sensitive and resistant *S. aureus* nasal colonisation in healthcare workers in a tertiary hospital**

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Objectives: The aim of this study was to establish the rates of nasal carriage in medical and nursing personnel of a tertiary care Hospital for both meticillin-sensitive (MSSA) and resistant (MRSA) *S. aureus*, in order to perform infection control measures, if necessary. The MRSA strains isolated were further tested by PCR for the presence of mecA and pvl genes.
Methods: A total of 220 nasal swabs were taken from 111 healthcare workers participating in the study, of which 43 were medical (21 ICU clinicians and 22 medical microbiology doctors) and 68 non-medical staff (34 nursing and 34 laboratory personnel). All swabs were cultured onto Columbia Blood agar (Oxoid, UK) and ChromID MRSA agar (Biomerieux, France). All unique-patient *S. aureus* isolates were identified by VITEK 2 automated system and were further tested for antibiotic susceptibility by both VITEK 2 system as well as Kirby-Bauer disk diffusion method. All *S. aureus* isolates were further tested for the presence of mecA and pvl genes by PCR (Genotype *Staphylococcus*, Hain Germany).
Results: In total, the prevalence of *S. aureus* nasal carriage was 18.0%. MSSA carriage was 12.6% and MRSA 5.4%.
MSSA prevalence was 11.6% (5/43) among medical personnel and, in particular, 9.1% (2/22) for laboratory doctors and 13.6% (3/21) for ICU clinicians. MSSA prevalence among non-medical staff, was 13.2% (9/68) and in particular, 17.6% (6/34) for laboratory personnel and 8.8% (3/34) for ICU nursing personnel.
MRSA prevalence was 4.7% (2/43) among medical personnel, and in particular, 4.5% (1/22) for laboratory doctors and 4.7% (1/21) for ICU clinicians. MRSA prevalence among non-medical staff was 5.9% (4/68), 0% for laboratory personnel and 11.8% (11/34) for ICU nursing personnel.
All MRSA strains were found positive for mecA gene, by PCR, while only one strain was found to be pvl positive (5%, 1/20).
Conclusion: The prevalence of MSSA as well as MRSA nasal carriage is within the expected rates, as commonly mentioned in the literature. The highest MSSA rates were found among non-medical laboratory staff, while, the highest MRSA rates were found among ICU nursing personnel. Among medical personnel, the prevalence rates were similar, for both MSSA and MRSA. This study is a part of an ongoing epidemiological surveillance conduced in our Hospital, in order to eliminate MRSA carriage.

P657 Meticillin-resistant *Staphylococcus aureus* nasal carriage in an army recruits population: prevalence and genotyping

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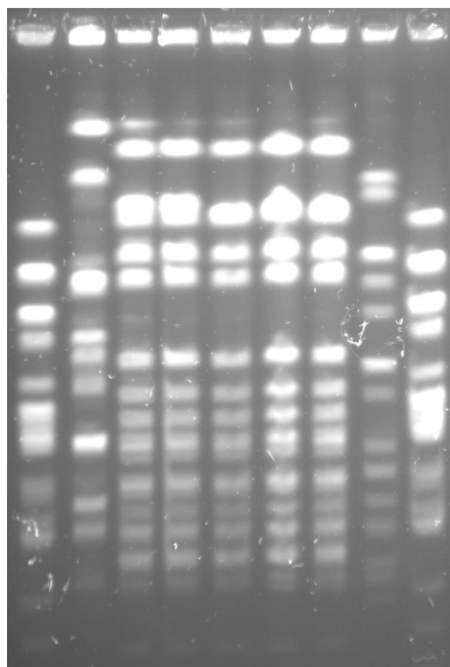
Objective: Although MRSA strains are strictly related to hospital setting community-acquired meticillin-resistant *Staphylococcus aureus* (CA-MRSA) is emerging worldwide. MRSA carriage is important for dissemination of CA-MRSA infections. The aim of this study was to determine the prevalence of MRSA nasal carriage among army recruits who are from different geographic areas of the country.
Methods: A total of 4843 army recruits were examined by nasal cultures. *Staphylococcus aureus* strains were characterised and identified by conventional methods. Antimicrobial susceptibilities were tested by disk diffusion method and all MRSA strains were typed by SCCmec and PFGE. Potential risk factors for colonisation have been noted down using a questionnaire; such as admissions to hospital, antibiotics usage, hometown, educational level, annual salary, job.



Results: *Staphylococcus aureus* strains were isolated from 552 (12%) of 4843 participants. Seven out of 552 *S. aureus* were resistant to meticillin. Overall community-associated MRSA carriage rate was 1.3%. Presence of mecA gene was confirmed in all 7 strains by mecA PCR. SCCmec typing and PFGE typing showed that 5 isolates were type III and A pulse-type, and the remaining 2 strains were type II and B and C pulse-types. The cases are found out to be admitted to hospital and/or used antibiotics.
Conclusion: MRSA is emerging in community level. Army is one of the risk group for MRSA dissemination and infection. The carriage level was 1.3% similar to carriage rates in previous studies from Turkey. The major A clone obtained from 5 of 7 soldiers were related to a hospital clone in a military hospital in the same area. Large scale studies should be done to evaluate presence a CA-MRSA clone other than hospital clones.

P658 Genotypic characterisation of *Staphylococcus lugdunensis*

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Background: *Staphylococcus lugdunensis* is a coagulase-negative staphylococcal species. Until the 90s, association of these bacteria to human pathogenic processes was considered a rare clinical event but during last years infections by this agent have considerably increased and now it has to be considered as a possible cause of breast abscesses, peritonitis, infected joint prostheses, osteomyelitis, discitis, septic arthritis and pacemaker infections.
Objectives: The main goal of this study was to perform the genotypic characterisation of *S. lugdunensis* clinical isolates recovered by the Microbiology Service of the Hospital Universitario Ntra. Sra. de Candelaria (Tenerife, Spain) during an eight year surveillance period (1999–2007).
Methodology: Seventy-five isolates recovered from 70 patients were firstly identified by classic microbiology biochemical and phenotypic methods. Identification was then confirmed by sequencing 16S rDNA and rpoB genes encoding the beta-subunit of RNA polimerase. After identification, each isolate was analysed by the following methods: (i) Multiplex PCR (MPCR) detection of the genes mecA and ileS-2, encoding meticillin and high-level mupirocin resistance, respectively; (ii) PCR amplification of the ribosomal internal spacer transcript (ITS-PCR), and (iii) Pulsed-Field Gel Electrophoresis macrorestriction patterns (RFLP-PFGE).
Results: MPCR amplification of the genes mecA and ileS-2 was negative for all the 75 isolates. ITS-PCR patterns permitted to distinguish 18 different genotypes, being types D and E the two major ones constituting the 25.3 and 18.6% of the isolates, respectively. The RFLP-PFGE analysis showed higher discriminative power than ITS-PCR and allowed to distinguish 34 genotypes. PFGE type 11 was the one including the higher number of isolates (8, 10.6%).
Conclusions. This study permits to conclude the existence of a high genotypic diversity within the species *S. lugdunensis*. We may conclude that the heterogeneity observed is greater than previously reported.

P659 Non-invasive coagulase negative staphylococci: population variability and meticillin resistance

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Objectives: Staphylococcal chromosomal cassette mec (SCCmec) is a large heterologous mobile genetic element carrying the mecA gene. This gene is the central element of meticillin resistance (MR) in staphylococci and has largely been studied in *Staphylococcus aureus*. The origins, reservoirs and dissemination routes of SCCmec elements are not well understood, although their elucidation might improve infection control measures targeting reduction of β-lactam resistance. For that purpose, it is necessary to characterise SCCmec elements circulating in staphylococci other than *S. aureus*, both in the hospital and community settings.
Methods: Five-hundred and twenty eight non-invasive coagulase negative staphylococci (CNS) were collected among 67 Danish volunteers from three different patient groups: (i) children $\leqslant$ 2 years; (ii) patients from a Dermatology department; and (iii) patients previously identified as community-onset MR *S. aureus* (CO-MRSA) carriers. Susceptibility tests for a panel of eight antibiotics were performed using disc diffusion. Internal transcribed spacer PCR (ITS-PCR) was performed in order to confirm VITEK 2 identification and to clarify unspeciated isolates.
Results: MR-CNS was found associated to patients from the dermatology department and to children $\leqslant$ 2 years (50% resistant isolates in these groups versus 4% MR-CNS isolates in CO-MRSA carriers). Fifteen different species were identified among the 528 CNS, out of which 15 (3%) could not be identified by VITEK 2. ITS-PCR was useful for the speciation of more exotic CNS isolates.
Conclusion: Resistance patterns showed that reservoirs of MR-CNS were associated with patients with hospital contact rather than CO-MRSA patients. In addition, the reservoirs of SCCmec elements among CNS isolates were more diverse than expected, since MR was observed in 11 of the staphylococci species recovered: *S. epidermidis* (40%), *S. haemolyticus* (74%), *S. hominis* (42%), *S. saprophyticus* (44%), *S. simulans* (20%), *S. xylosus* (25%), *S. warneri* (4%), *S. capitis* (45%), *S. cohnii* (50%), *S. caprae* (14%), and *S. kloosii* (20%). The ongoing

characterisation of the SCCmec elements in this collection will help to elucidate the genetic relationships and dissemination routes of these mobile genetic elements between *S. aureus* and CNS isolates and among CNS isolates.

P660 MRSA carriage in healthcare personnel in contact with farm animals

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Objective: In the Netherlands, veterinarians in contact with pigs and pig farmers were found to have a higher risk of meticillin resistant *S. aureus* (MRSA) carriage than the general population. Isolates of closely related spa-types, corresponding to MLST type ST398 were found in pig farmers, pig veterinarians and pigs. The objective of this study was to investigate if contact with pigs and veal calves or other livestock is a risk factor for MRSA carriage in Dutch healthcare workers (HCW).
Design: Healthcare workers in four general hospitals and one university hospital were asked to fill in questionnaires about contact with animals and take MRSA cultures of their throat and nares. Cultures of all HCW in contact with livestock were processed and for each HCW in contact with pigs or veal calves, screening cultures from a minimum of two HCW with no contact with livestock were processed.
Results: 77 of 1721 HCW (4.4%) reported direct or indirect contact with pigs and/or veal calves, 145 reported contact with other livestock animals. Two MRSA-positive HCWs were found; one in contact with veal calves and one in the control group. The MRSA carriage rate in the group in contact with pigs and veal calves was 1.7% (95% CI, 0.07%-8.0%) and in the control group (category 3) 0.15% (95% CI, 0.01–1.0%). No carriers were found among HCW in contact with other livestock (95% CI 0–3.2%).
Conclusions: An estimated 3% (CI 2.6–4.1%) of hospital staff working in Dutch hospitals with a rural adherence region belong to a high risk group for MRSA carriage according to the Dutch guidelines. While MRSA carriage in HCW in contact with livestock is higher (10-times) than in other HCW the difference is not statistically significant.

Networks for surveillance of antimicrobial resistance

P661 Five years of Belgian MIST (meropenem information on bacterial susceptibility testing) surveillance: 2001–2005

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Objectives: The activity of meropenem and 6 comparators was studied in the MIST trial, on a total of 2.553 isolates (706 Gram-positives and 1.847 Gram-negatives) from 6 general non teaching hospitals in Belgium, collected from 2001 to 2005.
Materials and Methods: Centres collected consecutively up to 50 and 75 unduplicated, aerobic Gram+ and Gram– isolates respectively from patients admitted in ICU, haematology and general wards. CLSI methods and E-tests were used for susceptibility testing. ESBL and AmpC production were confirmed by at least a ⩾3log2 reduction in ceftazidime MIC in the presence of clavulanate and a ceftazidime MIC > 8mg/l not lowered by the presence of clavulanate respectively.
Results: *S. aureus* (n = 237) was the most frequently isolated Gram+ bacteria with 98–100% susceptibility for the carbapenems. Among the Enterobacteriaceae (n = 1371), meropenem was the most active antibiotic (99.9%), followed by imipenem (98.5%), cefepime (98.3%) and amikacin (93.1%). Lower susceptibility rates were observed for piperacillin–tazobactam (83.1%), ceftazidime (80.2%) and ciprofloxacin (75.8%). More than 50% of *E. aerogenes* isolates (n = 232) were resistant to ceftazidime, piperacillin–tazobactam and ciprofloxacin, while the carbapenems and cefepime were extremely active (>95% susceptibility). Susceptibility rates among all Enterobacteriaceae remained stable for all antibiotics tested between 2001 and 2005. The prevalence of ESBL-producing isolates varied from 1.7 to 3.9% for all Enterobacteriaceae, from 0 to 4.3% for *E. coli* (n = 322), 0 to 9.5% for *K. pneumoniae* (n = 160) and 0 to 4.9% for *E. aerogenes*. The prevalence of AmpC producers among *E. aerogenes* isolates varied from 0 to 42%. Susceptibilities of ESBL and AmpC producers to carbapenems varied between 98–100%.
Against *P. aeruginosa* isolates (n = 273), the rank order of susceptibilities during the study period was: meropenem (88.3%), ceftazidime (87.9%), piperacillin–tazobactam (86.1%), amikacin (85.7%), imipenem (84.2%), cefepime (77.9%) and ciprofloxacin (76.9%). Susceptibility rates for *P. aeruginosa* remained stable for all antibiotics tested, excepted cefepime, which declined between 2001 and 2005.
Conclusions: During the study period, the carbapenems and cefepime were the most active agents against the Enterobacteriaceae, including ESBL and/or AmpC producers. Meropenem and ceftazidime were the most active agents against *P. aeruginosa*.During 5 years of Belgian MIST surveillance, susceptibility rates for meropenem remained stable.

P662 Bacterial resistance surveillance in China: a report from Mohnarin, 2004–2005

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Objective: To establish the nationwide antimicrobial resistant investigation network and get bacterial resistance information in China.
Methods: The clinical bacterial isolates were collected from 17 network hospitals around China, antibacterial minimal inhibitory concentration (MIC) were determined by standard agar dilution method recommended by CLSI.
Results: A total of 4075 isolates were collected from 17 hospitals in 15 cities. The results of MICs showed the followings: The detection rates of oxacillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis* were 62.9% and 82.89% respectively. Penicillin non-sensitive *Streptococcus pneumoniae* were 40.7%, which included 10.5% penicillin-resistant strains and 30.2% penicillin-intermediate strains 40.7%. 5 strains of Enterococci were vancomycin-intermediate, but all the strains were sensitive to teicoplanin. All Staphylococci were susceptible to glycopeptides. Macrolide-resistance was a predominant phenomenon for Gram-positive cocci. Enterobacteriaceae were obviously resistant to the third generation cephalosporin except for ceftazidime, and the resistance rates were 20%-70%. Carbapenems still remained highly activity against all the target bacteria. In addition, latamoxef, piperacillin / tazobactam, cefoperazone / sulbactam and cefepime were all active against Enterobacteriaceae and the resistant rates of which was bellow 10%. 10.6% of *Pseudomonas aeruginosa* and 10.4% of *Acinetobacter baumannii* were resistant to imipenem, and most of them were multidrug resistant. Combinations of β-lactam/β-lactamase inhibitor and fluoroquinolones also had potent antibacterial activity against non-fermenters. Amikacin was active against Enterobacteriaceae and *P. aeruginosa*.
Conclusion: meticillin-resistant Staphylococci, penicillin non-sensitive *S. pneumoniae*, macrolides-resistant Gram-positive cocci, cephalosporin-resistant Enterobacteriaceae, multidrug resistant nonfermenters and fluoroquinolone-resistant *E. coli* were the predominant problems. No glycopeptides-resistant *Staphylococcus* was found and glycopeptides-resistant Enterococci remained seldom.

P663 Resistance surveillance of bacterial pathogens from North American patients hospitalised with pneumonia: a 10-year report from the SENTRY Program (1997–2006)

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Objectives: The emergence of resistance (R) among pneumonia pathogens has resulted in increasing reliance upon fluoroquinolones and

advanced-generation β-lactams, including carbapenems. We summarize the prevalence and susceptibility (S) profiles of bacterial pneumonia pathogens collected during ten years of the SENTRY Antimicrobial Surveillance Program (1997–2006).

Methods: Participating North American medical centres (⩾24/year) referred 50 consecutive, non-duplicate pathogens (19,406 isolates) from lower respiratory tract sites determined to be the probable cause of pneumonia in hospitalised patients (community and nosocomial in origin). Isolate identifications were confirmed and susceptibility testing was performed using CLSI reference methods at a central laboratory (JMI Laboratories, North Liberty, IA).

Species (no. tested)	Antimicrobial agent	%R 1997	2002	2006
SA (5774)	OXA	39.4	45.9	61.4
PSA (3764)	CAZ	19.0	12.6	17.4
	IMP	8.0	7.3	13.6
	CIP	15.3	23.8	23.0
KSP (1541)	CRO	1.2 (9.3)	6.9 (21.7)	5.3 (12.6)
	IMP	0.0	0.4	3.2
	CIP	3.2	5.8	9.5
SPN (1282)	PEN	9.2	16.4	11.8
	ERY	14.5	23.7	29.4
EBS (1222)	CRO	13.2	7.8	17.9
	CIP	3.4	3.9	7.7
EC (798)	CRO	1.6 (7.0)	1.0 (7.2)	7.7 (7.7)
	CIP	1.6	9.3	30.8
ASP (540)	CAZ	29.5	39.0	58.8
	IMP	3.3	5.1	14.7
	CIP	34.4	54.2	73.5

*Numbers in parentheses are ESBL-phenotype rates (% ⩾2 μg/ml).

Results: Selected pathogens with unique or emerging R characteristics are in the Table. The 10 ranked pathogens comprised 90.4% of all isolates and included: *S. aureus* (SA; 28.1%) > *P. aeruginosa* (PSA; 19.4%) > *Klebsiella* spp. (KSP; 7.9%) > *H. influenzae* (7.7%) > *S. pneumoniae* (SPN; 6.6%) > *Enterobacter* spp. (6.3%) > *E. coli* (EC; 4.1%) > *Serratia* spp. (3.8%) > *S. maltophilia* (3.6%) > *Acinetobacter* spp. (ASP; 2.8%). Among Gram-positive species, R emergence was notable for SA (oxacillin [OXA] -R currently 61.4%; ERY-non-susceptibility [NS], 69.2%) and SPN (ERY-R, 29.4%; PEN-R, 11.8%). Among enterics, CIP-R (30.8% for EC in 2006) and ESBL-phenotype rates (KSP and EC; ranges 6.7–23.9% and 3.8–11.7%, respectively) have increased considerably. An increase in IMP-R in KSP primarily results from a continued east coast epidemic of clonal strains expressing serine carbapenemase (primarily KPC-2) enzymes. While CAZ- and IMP-R among PSA has increased, R to CAZ and fluoroquinolones have become predominant among ASP; only polymyxins remain largely S (>97%).

Conclusions: Although temporary resistance declines were detected among some North American pneumonia pathogens, all showed increasing resistance to most class agents during the monitored period. Continued longitudinal comparisons of emerging pathogens and changing susceptibility profiles are critical elements in guiding empiric therapies and epidemiologic interventions.

P664 Escalating resistance among non-fermentative Gram-negative bacilli: Report from the North American SENTRY Antimicrobial Surveillance Program (1997–2006)

H. Sader, T. Fritsche, M. Stilwell, R. Jones (North Liberty, US)

Objective: Systemic infections due to non-fermentative Gram-negative bacilli (NFB) are often difficult to treat because of rising resistance rates and limited therapeutic options. We evaluated the occurrence and antimicrobial susceptibility profiles of NFB pathogens collected over 10 years of the SENTRY Antimicrobial Surveillance Program in North America.

Methods: Consecutive clinically significant and non-duplicate NFB strains (12,435 total) were submitted from medical centres in the USA (⩾25) and Canada (5; 1997–2002 only) and susceptibility tested by broth microdilution using CLSI methods and interpretive criteria at a central laboratory (JMI Labs, North Liberty, Iowa). Specifically, changes in susceptibility rates were analysed over time. Carbapenem-resistant *P. aeruginosa* (PSA) and *Acinetobacter* spp. (ASP) were screened for metallo-β-lactamase (MBL) enzyme production by a disk approximation method or by Etest (AB BIODISK, Solna, Sweden).

Organism (no. tested)	MIC_{50} (μg/ml)/% susceptible[a] Amikacin	Ceftazidime	Imipenem	Levofloxacin	TMP/SMX
Acinetobacter spp. (1,779)	⩽4/84	8/59	0.25/91	⩽0.5/62	⩽0.5/74
Aeromonas spp. (102)	⩽4/97	⩽1/95	0.5/97	⩽0.5/99	⩽0.5/87
A. xylosoxidans (118)	>32/14	4/86	2/92	2/57	⩽0.5/90
B. cepacia (88)	>32/10	4/74	4/51	2/60	⩽0.5/88
P. aeruginosa (8,420)	⩽4/96	2/83	1/87	⩽0.5/74	>2/46
P. fluorescens/putida (155)	⩽4/96	4/90	1/87	⩽0.5/85	2/52
S. maltophilia (1,309)	>32/13	8/54	>8/1	1/83	⩽0.5/99

[a]Based on CLSI breakpoints for *Acinetobacter* spp., *P. aeruginosa* and *S. maltophilia*.
TMP/SMX = trimethoprim/sulfamethoxazole.

Results: The most commonly recovered non-fermentative Gram-negative bacilli were PSA (68%), ASP (14%) and *S. maltophilia* (SM; 11%). Polymyxin B (tested 2001–2006) was very active against PSA and ASP (⩾99% susceptible), but displayed more limited activity against other NFB. Tigecycline (2002–2006) exhibited good activity (MIC50 / % at ⩽2 mg/L) against ASP (0.5/92) and SM (0.5/93), but limited potency against PSA. The activities of other antimicrobials are in the Table. Susceptibility patterns of PSA and SM remained very stable during the decade. In contrast, ASP susceptibility rates decreased for most agents, including (1997/2006 % susceptible): imipenem (94/78), amikacin (92/80), gentamicin (77/61) and ciprofloxacin (73/53). Many imipenem-non-susceptible ASP strains (77%) were from three medical centres in the New York City area, the remaining were from 13 centres and collected mainly in 2006 (61%). Imipenem-resistant PSA strains were widely distributed geographically. No MBL-producing strain was identified among ASP and PSA isolates.

Conclusions: Therapeutic options to treat NFB infections are becoming increasingly limited secondary to progressively rising resistance rates. Continued NFB surveillance remains necessary to optimise empiric antimicrobial therapy, especially for the less frequently isolated and difficult to test species.

P665 Trends in prevalence and antimicrobial susceptibilities among skin and skin structure infection pathogens in North America: Report from the SENTRY Program (1997–2005)

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Objectives: Rising resistance (R) rates being observed globally in skin and skin structure infection (SSSI) pathogens are challenging accepted approaches to empiric management. We present a nine year summary of the prevalence and susceptibility (S) trends of bacterial pathogens producing SSSI collected from USA and Canadian medical centres participating in the SENTRY Antimicrobial Surveillance Program.

Methods: Participating North American (NA) medical centres were directed to send 50 consecutive, non-duplicate SSSI pathogens/year. USA (⩾21) and Canadian (5) medical centres submitted isolates for the years 1997–2002; USA sites only participated during 2004–2005. Isolate identifications were confirmed and susceptibility testing was performed using CLSI reference methods at a central laboratory (JMI Laboratories, North Liberty, IA).

Results: The top 7 ranked pathogens comprised 85.2% of the total (8,520 isolates; see Table) with *S. aureus* (SA) being predominant, ranging from 40.4–53.1% between years. Other ranking pathogens included *P. aeruginosa* (PSA), *Enterococcus* spp. (ENT), and *E. coli* (EC) which were second to fourth each year except in 2004, when ENT and PSA reversed rank. Prevalence of beta-haemolytic streptococci (BHS) varied from fifth in 1998, ninth in 2000, then fifth again in 2004/2005. The all-years NA MRSA rate was 35.9% (9% in Canada) and ranged from 31.3 to 56.2% among USA census zones. Highest ENT vancomycin R (VAN-R; 16.5%) was found in 2005 and highest erythromycin R rate (25.8%) in BHS was found in 2004. Trending increases in fluoroquinolone (FQ) R rates among EC and *Klebsiella* spp. (KSP) reached 24.4 and 12.8%, respectively. ESBL phenotype rates for EC (12.8%) peaked in 2004 and for KSP (20.5%) in 2005. In contrast to these changes, ceftazidime-, imipenem- and levofloxacin-R rates for PSA (higher in earlier years) have trended downwards.

Organism (no.)	R pattern	% R All years	% R (range)
SA (3,862)	MRSA	35.9	24.0–49.4
PSA (908)	Imipenem (IMP)-R	6.1	1.1–9.8
	Ceftazidime (CAZ)-R	10.0	7.8–13.1
	Levofloxacin (LEV)-R	18.9	15.7–22.5
ENT (748)	Vancomycin (VAN)-R	12.4	7.6–16.5
EC (610)	CAZ-R	2.8	0.0–7.0
	LEV-R	9.3	2.4–24.4
EBS (409)	CAZ-R	17.8	7.6–25.5
BHS (379)	Erythromycin (ERY)-R	19.8	8.8–25.8
KSP (344)	CAZ-R	5.2	0.0–17.9
	LEV-R	3.2	1.4–12.8

Conclusions: SSSI pathogen prevalence has changed minimally since 1997 (exception, BHS). MRSA rate differences are notable between countries and between USA census zones. R rates for ENT (VAN-R), and EC and KSP (FQ and ESBLs) are increasingly of concern whereas R among key agents targeting PSA have improved from earlier SENTRY surveillance periods. Continued surveillance monitoring of these trends, both locally and globally, provides useful information for empiric management of SSSI and in assessing needed changes to antimicrobial therapy guidelines.

P666 Meropenem, β-lactam with the highest activity against Gram-negative isolates from the intensive care unit – Part of the MYSTIC (Meropenem Yearly Susceptibility Test Information Collection) Programme, 1997–2007

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Objectives: MYSTIC (Meropenem Yearly Susceptibility Test Information Collection) is a longitudinal surveillance study comparing the in vitro activity of meropenem (MEM) and other antibiotics against bacterial strains from clinical units that use MEM for treatment. The aim of this analysis was to assess the in vitro activity of MEM and eight other antibiotics against Gram-negative isolates from a paediatric ICU.
Methods: 1300 Gram-negative isolates were obtained from various clinical specimens of children hospitalised in the ICU in 1997–2007. The isolates were identified using conventional methods. The Minimum Inhibitory Concentrations (MICs) of MEM, imipenem (IPM), piperacillin+tazobactam (TAZ), cefotaxime (CTX), ceftazidime (CAZ), cefepime (CPE), gentamicin (GM) and ciprofloxacin (CIP) were determined using the NCCLS agar dilution method.
Results: The collection of Gram-negative isolates included *Escherichia coli* (n = 187), *Enterobacter cloacae* (n = 233), *Klebsiella oxytoca* (n = 118), *Klebsiella pneumoniae* (n = 211), *Serratia marcescens* (n = 50), *Acinetobacter baumannii* (n = 137), *Pseudomonas aeruginosa* (n = 253) and other species (n = 111). MEM, IPM and CIP were the most active against 94.2%, 92.2% and 95.2% of isolates, respectively. The MIC90 (mg/L) of MEM was nearly identical in 1997 and 2007. It was equal to 0.06 for Enterobacteriaceae, 1.0 for *A. baumanii* and 8.0 for *P. aeruginosa*. The MIC90 of IPM was equal to 0.25 for Enterobacteriaceae, 1.0 for *A. baumannii* and 16 for *P. aeruginosa*. During 1997–2007, among Enterobacteriaceae were only found two *E. cloacae* and one *E. amnigenus* resistant to carbapenems. Among carbapenem resistant *P. aeruginosa* isolates one strain producing metallo-β-lactamase VIM4 was found. The overall order of susceptibility of tested isolates to other β-lactams was CPE (85.8%) > TAZ (75.2%) > CAZ (72%) > CTX (53.6%). Susceptibility to GM characterised 66.9% of isolates. The incidence of extended spectrum β-lactamase producers among Enterobacteriaceae decreased from 39.7% in 1997 to 20.7% in 2007.
Conclusions: MEM, IMP and CIP were the most active antibiotics (>90% susceptibility) against Gram-negative isolates, with no observed reduction in activity over 11 years. A greater proportion of *P. aeruginosa* isolates were susceptible to MEM than to IPM. Carbapenems, such as MEM, can be used as an option in the first line therapy of nosocomial infections in the ICU.

P667 *Acinetobacter* spp. infections: the Latin American scenario in the SENTRY Antimicrobial Surveillance Program (1997–2006)

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Objectives: To evaluated the frequency of occurrence, the antimicrobial susceptibility profile and the temporal evolution of antimicrobial resistance among *Acinetobacter* spp. isolated in Latin America. *Acinetobacter* spp. is an important nosocomial pathogen in Latin America and exhibits high rates of acquired resistance to many antimicrobial agents.
Methods: Consecutive non-duplicate bacterial pathogens were collected in the last decade (1997–2006) from hospitalised patients in 10 Latin American medical centres (7 countries). The antimicrobial susceptibility profile to >20 antimicrobial agents was evaluated by CLSI broth microdilution methods and interpreted by CLSI M100-S17 (2007) or US-FDA package insert criteria for Enterobacteriaceae (tigecycline).
Results: A total of 1,807 isolates were collected during the 10-year period. The highest number of isolates were collected from Brazil (53%) > Argentina (19%) > Chile (14%) and Mexico (6%). Most isolates were collected from bloodstream (57.7%) followed by respiratory tract (31.9%) infections. The activities of selected drugs are shown in the table. Polymyxin B, tigecycline and imipenem demonstrated the highest in vitro activity against *Acinetobacter* spp. A significant increase ($p < 0.05$) in the resistance rates for imipenem (6.7–24.4%), meropenem (6.7–24.3%), cefepime (38.3–54.8%), ceftazidime (56–72.1%), amikacin (55.4–68.2%) was observed when comparing 1997 and 2006 results.

Antimicrobials	MIC (mg/L)		% Susceptible	% Resistant
	MIC_{50}	MIC_{90}		
Amikacin	>32	>32	34.9	59.5
Cefepime	16	>16	35.1	49.7
Ceftazidime	>16	>16	27.9	63.7
Ciprofloxacin	>2	>4	30.5	69.0
Imipenem	1	>8	83.5	15.3
Meropenem	2	>8	81.0	15.3
Polymyxin B	0.5	1	98.1	1.9
Tigecycline[a]	0.5	2	98.1	0.0

a. Not tested in all years.

Conclusions: Polymyxin B remains the most active drug against *Acinetobacter* spp. in the Latin American countries evaluated by the SENTRY Program and tigecycline was also active. An important reduction in the susceptibility rates to some broad-spectrum antimicrobials, including the carbapenems, was observed, limiting therapeutic options available.

P668 Drug resistance amongst European isolates of *Escherichia coli* isolated from the MYSTIC Programme 2007

P. Turner (Macclesfield, UK)

Objectives: In a time where resistance amongst the Enterobacteriaceae tends to be dominated by *Klebsiella* and *Enterobacter* species it is useful to also examine the susceptibility patterns expressed by *Escherichia coli* – still the commonest Gram-negative organism involved in nosocomial infections.
Methods: During 2007 a total of 496 clinically significant, non-copy isolates of *E. coli* were obtained from European centres involved in the MYSTIC (Meropenem Yearly Susceptibility Test Information Collection) Programme. These were examined using CLSI methodology and associated interpretive criteria in order to ascertain their susceptibility to a range of compounds. Extended-spectrum β-lactamase (ESBL) producers were confirmed by synergy with clavulanic acid and AmpC production by lack of synergy with this compound.
Results: See the table.

		% Susceptible*						
E. coli	n	MEM	IPM	CAZ	CPM	P+T	CIP	GM
All	496	99.8	100	84.9	90.6	88.7	76.4	84.6
ESBL	29	100	100	0	0	65.5	31	30
AmpC	13	100	100	0	61.5	53.9	61.5	100
R to CAZ/CIP	25	96	100	0	30	56	0	28.6
R to CAZ/CIP/GM	10	90	100	0	42.9	50	0	0

*CLSI criteria.
MEM: meropenem; IPM: imipenem; CAZ: ceftazidime; CPM: cefepime; P+T: piperacillin+tazobactam; CIP: ciprofloxacin; GM: gentamicin.

Conclusions: Whilst still at a low level ESBL (0.6%) and AmpC (0.3%) positive strains were isolated, and these were frequently also resistant to other classes of antibiotics (fluoroquinolones, aminoglycosides and β-lactam/β-lactamase combinations) as well as the cephalosporins. The carbapenems remain a useful therapy option against this type of isolate.

P669 Antimicrobial drug resistance in human non-typhoidal *Salmonella* isolates in Europe 2000–04: a report from the Enter-net international surveillance network

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Objectives: To investigate the occurrence of resistance to antimicrobials in salmonella strains from humans in ten European countries over the five year period 2000–2004
Methods: The antimicrobial susceptibility test results of salmonella isolates received from ten countries – Austria, Denmark, England and Wales, Germany, Ireland, Italy, Luxembourg, The Netherlands, Scotland and Spain – that supplied data for all years between 2000 and 2004 were extracted from the Enter-net *Salmonella* database. Interpretation of sensitivity results had previously been harmonised throughout the Enter-net network. Descriptive and statistical analyses were undertaken using Microsoft Excel and Epi Info version six. Relative proportions were compared using the Chi-squared test for trend.
Results: Results of antimicrobial susceptibility testing for 134,310 isolates of non-typhoidal salmonellas from cases of human infection in ten European countries from 2000–04 has demonstrated an overall increase in the occurrence of resistance, from 57% to 66% over the period of study. In contrast multiple resistance (to four or more antimicrobial drugs) has declined from 18% to 15%. The most significant increase in resistance has been to nalidixic acid (14% to 20%), particularly in *Salmonella enterica* serovar Enteritidis (10% to 26%), the most common serovar over the period of study. For England and Wales this increase has for the most part been attributed to infections linked to contaminated eggs originating outwith the United Kingdom. For S. Typhimurium, the second most prevalent serovar, there has been an overall decline in the occurrence of resistance to ampicillin, chloramphenicol and tetracyclines, attributed to a decline in the occurrence of multiresistant S. Typhimurium DT 104. For S. Virchow, a serotype with a predeliction for invasive disease, there has been a substantive increase in resistance to most antimicrobials, attributed to the spread of drug-resistant strains associated with poultry.
Conclusion: Results indicate that the overall interpretation of susceptibility data from different countries ishould take into account both indigenous strains and also strains originating as a result of foreign travel and the importation of contaminated foods. Because of the widespread importation of foods, it is important that controls to reduce the emergence and spread of drug-resistant strains of *Salmonella* are internationally implemented.

P670 Evolution of antimicrobial susceptibility patterns of aerobic and facultative Gram-negative bacilli causing intra-abdominal infections: results from the SMART studies, 2003–2007

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Objective: SMART (Study for Monitoring Antimicrobial Resistance Trends) is an ongoing global antimicrobial surveillance programme focused on clinical isolates from intra-abdominal infections (IAI). The objective of this subanalysis was to assess the evolution of the antimicrobial susceptibility patterns among aerobic and facultative Gram-negative bacilli recovered over a period of 5 years at our participating institution.
Methods: We tested the in vitro activity of 10 antimicrobials, commonly used to treat IAI, against consecutive unique isolates from IAI using microdilution techniques according to the CLSI guidelines for MIC testing. All isolates were screened phenotypically for extended-spectrum β-lactamase (ESBL) production. Isolates recovered within 48 hours of hospitalisation were considered community-acquired (CA).
Results: Over the study period a total of 572 aerobic and facultative Gram-negative bacilli were recovered from 510 patients, of which 258 (45%) were CA. Enterobacteriaceae composed 91% of the total isolates. *E. coli* was the most common isolated species (52%). The susceptibility rates of Enterobacteriaceae over the study period ranged from 76.8–85.2% to the fluoroquinolones, 86.6–96.1% to cefotaxime, 91.1–100% to cefepime, 89–96.1% to piperacillin–tazobactam, 95.5–100% to amikacin, 98.2–100% to imipenem, and 98.2–100% to ertapenem. ESBLs were detected in 6.3% of *E. coli*, 5.4% of *Klebsiella* spp., and 2.7% of *Enterobacter* spp. ESBL producers generally had a more antibiotic-resistant profile than non-ESBL producers and 16% of them were CA. Susceptibility rates to fluoroquinolones, cefepime, piperacilin-tazobactam, amikacin, imipenem, and ertapenem were, respectively, for *P. aeruginosa*: 71.8%, 76.9%, 82%, 82%, 58.9%, and 28.2%; for *A. baumannii*: 66.6%, 66.6%, 66.6%, 66.6%, 100%, and 33.3%; and for *S. maltophilia*: 42.9%, 0%, 0%, 14.3%, 0%, and 0%.
Conclusions: Over the 5-year-study period we have not observed significant increases in resistance of aerobic and facultative Gram-negative bacilli causing IAI to commonly used β-lactam antimicrobial drugs. A minority of ESBL-producing Enterobacteriaceae were CA. The carbapenems, including group I agents like ertapenem, were the most reliably active drugs in vitro against isolates producing IAI.

P671 **Sentinel Surveillance of Antibiotic Resistance in Switzerland (SEARCH)**

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Objectives: Comprehensive, representative and valid surveillance of antibiotic resistance on local, national and international levels is key to resistance control. We established a national surveillance programme in Switzerland within the frame of the National Research Program of Antibiotic Resistance (NRP49).

Methods: Routine resistance data are collected electronically on a weekly base from 22 clinical microbiology laboratories into a central data base. Data are validated, mapped to a common nomenclature and subjected to algorithms identifying double samples, defining microorganisms as contaminants versus pathogens and nosocomial versus community-acquired.

Results: In 2006 SEARCH received resistance data on 100,988 clinical isolates and 396 different bacterial species. The system represents approximately 80% of acute care hospital days and >30% of Swiss practitioners in the outpatient setting. Geographical distribution is even. 12.2% of samples derive from children <15 years of age and 4.6% from <2 years olds.

Isolates from sterile anatomical sites contribute 24% of all data. The relative distribution of hospital isolates was 24.6% from outpatient departments, 17.2% from surgery, 16.6% from internal medicine, 8.1% from intensive care units, 5.5% from paediatrics, 4.4% from obstetrics and gynaecology and 23.6% mixed or others. Among practitioners providing isolates the specialities were 30.4% general practitioners, 20.2% gynaecologists, 14.9% internal medicine, 7.6% paediatricians and 26.9% others.

In 2006, 2305 Swiss hospitalised patients experienced colonisation or infection by at least on of four prominent multi-resistant germs (MDR) (Meticillin-resistant *S. aureus*, extended-spectrum β-lactamase producers, multi-resistant *P. aeruginosa* and *Acinetobacter* spp.) and 15.2% of these had invasive infection. Attributable mortality was at least 80 deaths per year. Prevalence of MDR showed significant geographical differences with higher rates in South-West Switzerland (6.6%) compared to the Northeast (3.8%, $p < 0.001$).

Conclusion: The Swiss national system for antibiotic resistance surveillance provides a valuable tool for large scale, detailed and timely monitoring of antibiotic resistance in human medicine.

P672 **Antimicrobial susceptibility patterns of inducible Enterobacteriaceae isolated from intra-abdominal infections worldwide: results from SMART 2006**

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Objective: SMART (Study for Monitoring Antimicrobial Resistance Trends) is an ongoing global antimicrobial surveillance programme focused on clinical isolates from intra-abdominal infections (IAI). We assessed antimicrobial susceptibility patterns among inducible Enterobacteriaceae worldwide in 2006.

Methods: 82 worldwide sites (distributed as: Asia/Pacific – 24; Europe – 34; Latin America – 11; Middle East/Africa – 5; North America – 8) tested the in vitro activity of 12 antimicrobial agents commonly used to treat IAI against consecutive unique inducible Enterobacteriaceae isolated from IAI. Microdilution techniques followed Clinical Laboratory Standard Institute (CLSI) guidelines. All *Enterobacter*, *Serratia*, *Citrobacter*, *Providencia* spp., *Morganella morganii*, *Hafnia alvei*, & *Proteus vulgaris* were considered for the purposes of this study to possess an inducible ampC β-lactamase gene.

Results: Inducible Enterobacteriaceae were recovered from 19% (1175/6134) pts, representing 18% (1204/6633) of total isolates. 38% (461) of isolates were recovered <48 hrs and 60% (721) were recovered ⩾48 hrs of hospitalisation; whereas, for 2% (22) the time recovered was unknown. *Enterobacter* spp. (48%), *Citrobacter* spp. (24%), *M. morganii* (11%), and *Serratia* spp. (7%) were the most common inducible Enterobacteriaceae. % susceptibility rates are reported below.

Conclusion: *Enterobacter* species were the most common isolates. Ampicillin-sulbactam and cefoxitin were the least active agents against inducible Enterobacteriaceae; whereas, imipenem, ertapenem, and amikacin were the most active agents in vitro.

	Enterobacter spp. N = 583	*Citrobacter freundii* N = 210	*Morganella morganii* N = 127	*Serratia* spp. N = 86
Ertapenem	93	98	99	99
Imipenem	98	98	98	99
Cefepime	85	91	97	94
Ceftriaxone	60	64	80	87
Cefoxitin	8	13	78	38
Ceftazidime	61	66	77	91
Cefotaxime	61	62	91	86
Ampicillin–sulbactam	21	43	4	13
Piperacillin–tazobactam	75	80	95	91
Amikacin	94	92	98	99
Ciprofloxacin	84	80	86	87
Levofloxacin	88	85	89	94

P673 **CARE-ICU – Controlling Antibiotic REsistance in Intensive Care Units – First report from a web-based programme for improved infection control in European ICUs**

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Background: CARE-ICU is a web-based programme for IC surveillance in the ICU setting developed within the EU Commission sponsored project IPSE. The aim of the programme is to improve surveillance and control of antibiotic resistance (ABR), AB consumption (ABC) and hygienic precautions in the ICU setting of EU member states.

Methods: A programme for yearly registration of ABC, resistance and clinical practices with automatic feedback through a website http://www4.smittskyddsinstitutet.se/careicu was developed. Participants are national ICU networks and individual ICUs. The national contact points of IPSE were asked to identify ICUs who would be willing to take part in the large pilot study. ABR was defined as the sum of intermediate susceptible and resistant strains. Antibiotics (AB) to which >90% of isolates of a species were susceptible were defined as treatment alternatives (TA90) which is a novel index of susceptibility to measure the magnitude of MDR among Gram-negative bacteria. For each ICU the clinical practices and infection control measures were analysed in relation to the needs based on local resistance patterns and ABC.

Results: 34 ICUs in 8 countries participated in the collection of ICU data for 2005. ABC varied widely from 348–4992 DDD1000 (defined daily dosages per 1000 occupied bed days), median 1417 DDD1000. Frequencies of ABR varied greatly between species, ICUs and countries: The median (range) of MRSA, ESBL phenotype of *E. coli*, ESBL phenotype of *K. pneumoniae* were in percentages: 11.6 (0–100), 3.9 (0–80) and 14.3 (0–77.8) respectively. TA90 for *P. aeruginosa* to aminoglycoside, ceftazidime, ciprofloxacin and carbapenem varied between 0 (9 ICUs), 1 (8 ICUs), 2 (3 ICUs), 3 (6 ICUs) and 4 (4 ICUs). Failure to screen for alert microorganisms in relevant patients at admission was a general problem as well as lack of rooms for isolation precautions and cohort care for patients colonised or infected with Alert organism. Understaffing was common but facilities for hand disinfection was in general available by each bed.

Conclusions: CARE-ICU has become a focal point for promoting more appropriate use of AB and improved infection control. By correlating ABC and resistance, as well as hygienic precautions, best practice in AB policy and hygiene interventions was defined for each unit. The single most needed improvement in almost all settings was increased hygiene compliance of healthcare workers.

P674 Antimicrobial susceptibility of the bacteria causing community-acquired respiratory tract infections in Poland, 2005–2006 (continuation of the Alexander Project)

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Objectives: The aim of the study was to evaluate susceptibility patterns of major agents of acute pharyngitis/tonsilitis and lower respiratory tract infections in Poland, 2005–2006.

Methods: The study was performed on 811 isolates of the four species: *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Streptococcus pyogenes* and *Moraxella catarrhalis*. The strains were isolated in 42 healthcare centres, from ambulatory or hospitalised patients. In the second case, the study included only the isolates, which were recovered during the first 48 hours after the patients' admission. Isolates were checked for their susceptibility to drugs commonly used in the treatment of respiratory tract infections, according to the CLSI guidelines. ATCC strains were used for the quality control purposes.

Results: Among pneumococci, more than 20% were non-susceptible to penicillin (PNSP), almost 70% of which were fully resistant. Nearly 90% of PNSP were also resistant to at least two other drug groups, e.g. macrolides and co-trimoxazole. Almost 10% of pneumococci were non-susceptible to the 3rd-generation cephalosporins. Beside penicillin, the lowest in vitro activity against *S. pneumoniae* had co-trimoxazole and tetracycline. Among *H. influenzae*, 8.1% were ampicillin-resistant β-lactamase producers. All of them were in vitro susceptible to amoxicillin/clavulanic acid and 3rd-generation cephalosporins. Co-trimoxazole and clarithromycin were the least active in vitro drugs against this species. More than 91% of *M. catarrhalis* isolates were penicillin-resistant β-lactamase producers, although all of them were susceptible to various other antimicrobials. All of the group A streptococci were susceptible to penicillin, however an increase in resistance to tetracycline and macrolides was observed.

Conclusion: Although the frequency of PNSP (~20%) seems to be stable in Poland, the number of isolates resistant to at least three groups of antimicrobials is being increased. Enzymatic resistance of *H. influenzae* to β-lactams is stable as well (~10%) of isolates, but resistance to macrolides is growing. Further increase in resistance of *S. pyogenes* to all groups of macrolides is being observed. *M. catarrhalis* remains susceptible to many antibiotics.

P675 Comparison of antimicrobial susceptibilities of bacteria isolated in Canada, the United States, and Mexico – Results from the T.E.S.T. Program 2004–2007

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Objectives: The Tigecycline Evaluation Surveillance Trial (T.E.S.T.) is a longitudinal surveillance study that monitors the in vitro activity of tigecycline (TIG) and several comparator antimicrobics in dozens of countries. This analysis of T.E.S.T. data compares the susceptibility of commonly-isolated pathogens collected in Canada, The United States of America, and Mexico.

Methods: 49,886 isolates were collected and identified to the species level at 231 participating sites (215 in the USA, 8 in Canada, and 8 in Mexico) and confirmed by the central laboratory. MICs were determined by each site using supplied broth microdilution panels and interpreted according to CLSI guidelines. % susceptible of each species or phenotype to each of the study drugs was determined for isolates from each of the 3 countries.

Results: Organism/antimicrobic combinations which showed more than 25% variation in % susceptible between countries are listed below:

Organism	Canada	USA	Mexico
Acinetobacter	n = 123	n = 3113	n = 95
Ak	91	83	56
Cpe	79	50	54
Caz	81	50	28
Lvx	81	52	35
PT	86	60	40
S. pneumoniae	n = 31	n = 1070	n = 70
Mer	94	79	66
H. influenzae	n = 132	n = 2749	n = 67
Amp	83	73	57
E. coli	n = 220	n = 6370	n = 238
Aug	76	76	37
Amp	47	43	8
Cpe	96	98	62
Cax	95	95	48
Lvx	77	75	29
Min	85	87	62
ESBL+	n = 16	n = 595	n = 126
Mer	100	73	99
PT	94	52	79

Ak = Amikacin; Cpe = cefepime; Caz = ceftazidime; Lvx = levofloxacin; PT = piperacillin/tazobactam; Mer = meropenem; Amp = ampicillin; Aug = amox/clav; Cax = ceftriaxone; Min = minocycline.

Conclusions: There are several pathogens for which antibiograms differ dramatically between Canada, the USA, and Mexico. Although the US sample size is vastly larger than the other two countries, there were enough strains to lend credence to the data from Canada and Mexico. Many drugs showed no significant difference in %S among the three countries, but when there were large variations, Mexico usually had the lowest susceptibility rates, and Canada the highest. Interestingly, the positions of Mexico and the USA were reversed for ESBL producers vs. Mer and PT.

P676 A global perspective of antimicrobial in vitro activity from the T.E.S.T. Program: annual analysis from 2004–2006

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Background: Tigecycline, the first member of the glycylcyclines, was marketed in mid 2005 and has demonstrated success against multiply-resistant species and phenotypes. Due to its chemical structure, resistance to tigecycline is reportedly difficult to produce even in the laboratory. The T.E.S.T. Program is an ongoing global surveillance with the first post-marketing prospective report of tigecycline and comparator in vitro activity for the years 2004 through 2006.

Methods: More than 60,000 clinical isolates were collected from 335 investigative sites in 47 countries worldwide. MICs were determined by broth microdilution according to CLSI guidelines using identical panels.

Results: Results are given by year for all pathogens and antimicrobials. Summary data for tigecycline and key species are presented in the table.

Conclusions: Tigecycline demonstrated no significant shift in MIC values over three years from its pre-marketing baseline values. Tigecycline activity was retained even against strains resistant to other antimicrobials, including ESBL-producers, *Acinetobacter* spp., meticillin-resistant *S. aureus*, vancomycin-resistant enterococci, and *S. pneumoniae*.

Organism	n (04/05/06)	2004		2005		2006	
		MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
E. co./K. pn./K. ox.[1]	5029/5298/5943	0.25	1	0.25	1	0.25	1
ESBL producers	370/447/569	0.5	2	0.5	2	0.5	2
Enterobacter spp.	2347/2315/2660	0.5	1	0.5	2	0.5	2
Serratia spp.	986/1000/1103	1	2	1	2	1	2
Acinetobacter spp.	1482/1342/1418	0.25	1	0.25	1	0.25	1
P. aeruginosa	2043/2098/2456	8	>16	8	>16	8	>16
S. aureus	2426/2604/2523	0.12	0.25	0.12	0.25	0.12	0.25
MRSA	1139/1234/1017	0.12	0.25	0.12	0.25	0.12	0.25
Enterococcus spp.	1483/1606/1830	0.06	0.12	0.06	0.12	0.06	0.12
VRE	208/247/286	0.06	0.12	0.06	0.12	0.06	0.12
S. pneumoniae	1329/1371/1418	0.06	0.5	0.03	0.25	0.03	0.12

[1]*E. coli*, *K. pneumoniae*, *K. oxytoca*.

P677 Prevalence of *Helicobacter pylori* resistance to antimicrobial agents at two different locations in Belgium: a 10-year surveillance

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Objectives: Monitoring of primary antibiotic resistance in *H. pylori* at a local level is of utmost importance since antimicrobial resistance is an important indicator of treatment failures. We aimed to compare the rates of primary antimicrobial resistance of *H. pylori* (Hp) at two locations in Belgium and to investigate possible links with disease severity, gender, age and ethnicity of patients (pts.).

Methods: Between 01/98 and 10/07, Hp culture of gastric biopsies from untreated pts. endoscoped at UCL St-Luc, Brussels (Br) and at UCL Mont-Godinne, Yvoir (Yv), south Belgium was carried out centrally at one laboratory. Susceptibility testing (disc diffusion and/or Etest) to the following agents was performed: metronidazole (Mtz), clarithromycin (Cla), amoxicillin (Amx), levofloxacin (Lvx) and tetracycline (Tet).

Results: Data were available for 1614 Hp isolates (977 in Brussels; 637 in Yvoir). The prevalence of Hp infection was 25% in B (36% non-caucasians) and 21% in Y (9% non Caucasians). Global primary resistance rates were 31.7% to Mtz; 18.4% to Cla and 16.8% to Lvx; 7.4% of the strains displayed double Mtz/Cla resistance and 2.9% displayed triple resistance (Mtz/Cla/Lvx). Resistance rate to Amox and Tet was T1%. The rate of Mtz resistance was higher in B than in Y (40% vs. 25%; $P < 0.05$) while it was identical for Cla (16.6% vs. 21.2%, NS) and for Lvx (15.5 vs. 19.0%; NS). Primary resistance to Mtz was significantly higher in non-Belgian born patients (41.1% vs. 27.8% in Belgian-born; $P < 0.01$) and in females (38.2% vs. 26.1% in males; $P < 0.05$). There was no difference in resistance between ulcer and non-ulcer dyspepsia-associated Hp isolates.

Conclusion: High Mtz, Cla and Lvx resistance rates were observed at two different locations in Belgium and no significant trends occurred over time, except for Lvx resistance which increases from 10% in 2002 to over 25% in 2007. Country of birth and female gender were the only risk factors identified for Mtz resistance. Our results emphasize the importance of local data for optimisation of anti-Hp treatment strategies.

P678 Potency and spectrum of tigecycline tested against bacterial pathogens producing skin and skin structure infections in European medical centres, including community-acquired meticillin-resistant *S. aureus*

H. Sader, R. Jones, G. Moet, T. Fritsche (North Liberty, US)

Objective: To evaluate the activity and potency of tigecycline when tested against a large collection of bacterial pathogens causing skin and skin structure infections (SSSI). Tigecycline is the sentinel representative of the glycylcycline class and was recently approved by the European Medicines Agency for the treatment of complicated SSSI and intra-abdominal infections.

Methods: Consecutive, non-duplicate bacterial isolates (4,567 strains) were collected from 2000 to 2007 from patients with documented SSSI in >30 medical centres (14 countries) participating in the Tigecycline Surveillance Program in Europe. All isolates were tested using CLSI broth microdilution methods against tigecycline and key comparator agents commonly used for therapy of SSSI. Tigecycline-susceptible (S) breakpoints (US-FDA/EUCAST) were defined as ⩽2/⩽1 mg/L for Enterobacteriaceae (ENT); ⩽0.5/⩽0.5 mg/L for staphylococci, and ⩽0.25/⩽0.25 mg/L for streptococci and enterococci. *S. aureus* infections diagnosed within the first 48 hours of hospitalisation were considered to be of community origin.

Results: SSSI pathogen rank order (top 8), potency (MIC90) and S rates for tigecycline are shown in the Table. *S. aureus* (22.7% MRSA) was isolated from 45.7% of SSSI cases and tigecycline inhibited 99.8% of strains, including all community-acquired (CA)-MRSA, at the S breakpoint. CA-MRSA showed low S to erythromycin (37.5%), clindamycin (63.5%) and levofloxacin (17.5%). Tigecycline was also very active against enterococci (93.2% S), including vancomycin-non-S strains (100.0% S), BHS (99.6% S) and CoNS (97.9% S). Linezolid was also active against Gram-positive organisms, but generally 8-fold less potent than tigecycline. Tigecycline (98.4–100.0% S) and imipenem (98.4–100.0% S) were the most active compounds tested against *E. coli*, *Klebsiella* spp. and *Enterobacter* spp., while high rates of R to levofloxacin (5.5–16.4%) and gentamicin (7.1–12.7%) were observed among these pathogens.

Organism (no. of isolates/% of total)	MIC_{90}/%S[a]				
	TIG	LEV	CLI	T/S	LZD
S. aureus (2,089/45.7)	0.25/99.8	>4/76.4	>2/88.5	⩽0.5/97.6	2/100.0
Meticillin-resistant *S. aureus* (MRSA; 474/10.4)	0.25/99.6	>4/11.0	>2/59.1	2/91.7	2/100.0
Community-acquired (CA)-MRSA (137/6.6)	0.25/100.0	>4/17.5	>2/63.5	1/94.9	2/100.0
E. coli (426/9.3)	0.25/99.5	>4/80.3	–/–	>2/70.1	–/–
P. aeruginosa (PSA; 399/8.7)	>4/–	>4/71.4	–/–	>2/6.3	–/–
Enterococci (293/6.4)	0.25/93.2	>4/59.0	–/–	–/–	2/100.0
Beta-haemolytic streptococci (BHS; 256/5.6)	⩽0.12/100.0	1/100.0	⩽0.25/92.6	⩽0.5/99.6	1/100.0
Coagulase-neg. staphylococci (CoNS; 234/5.1)	0.5/97.9	>4/49.6	>2/74.4	>2/69.3	1/100.0
Enterobacter spp. (ESP; 182/4.0)	1/98.4	1/92.3	–/–	>2/89.6	–/–
Klebsiella spp. (KSP; 142/3.1)	1/100.0	4/88.0	–/–	>2/81.0	–/–

a. According to US-FDA breakpoints. TIG = tigecycline; LEV = levofloxacin; CLI = clindamycin; T/S = trimethoprim/sulfamethoxazole and LZD = linezolid.

Conclusions: Tigecycline was highly active against the top 8-ranked pathogens producing SSSI, except for *P. aeruginosa*, and showed the broadest spectrum of activity among the antimicrobials tested. Tigecycline, linezolid and vancomycin were the most active compounds tested against Gram-positive species while tigecycline, amikacin and imipenem were the most active against Gram-negatives. Tigecycline represents a welcome choice for use in treating common Gram-positive and negative pathogens producing serious SSSI.

P679 Trends in bacteraemia: England 2002–2006

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Objectives: This paper presents the nine species of bacteria (and *Candida*) responsible for the majority of bloodstream infections (BSI) in England during 2006, and the changes in trends of these species over the last five years.

Methods: Voluntary reports of bacteraemia are collected using electronic reporting from laboratories in England and Wales to a national database. Information indicating how many blood cultures were taken and how many tested positive were provided through the mandatory MRSA surveillance undertaken in England. Bloodstream infections are defined as clinically-significant positive blood cultures.

Results: The total number of blood cultures taken has increased by over 17% between 2002 and 2006, while the number of cultures that prove positive has increased by 14%. The numbers of bloodstream infections reported has increased, however, this may in part be due to an increase in voluntary reporting. The top five organisms responsible for BSI have changed in relative frequency over the last five years, with *E. coli*

overtaking *S. aureus* rather dramatically as the most common cause of bacteraemia.

	2002	2003	2004	2005	2006
Total blood cultures	1500832	1611714	1546630	1695371	1752914
Total positive blood cultures	249311	264766	261899	278281	285152
All episodes of bloodstream infections	76231	92770	96101	102785	109696
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Escherichia coli	12709 (16.7)	15052 (16.2)	15741 (16.4)	16961 (16.5)	18043 (16.4)
Coagulase negative staphylococci	6604 (8.7)	8609 (9.3)	10091 (10.5)	11708 (11.4)	14184 (12.9)
Staphylococcus aureus	12895 (16.9)	14603 (15.7)	14173 (14.7)	14065 (13.7)	13616 (12.4)
Enterococcus spp.	4421 (5.8)	5611 (6.0)	5887 (6.1)	6477 (6.3)	7070 (6.4)
Klebsiella spp.	3515 (4.6)	4169 (4.5)	4639 (4.8)	4853 (4.7)	5162 (4.7)
Streptococcus pneumoniae	4163 (5.5)	5135 (5.5)	4526 (4.7)	4971 (4.8)	4539 (4.1)
Pseudomonas spp.	2382 (3.1)	2969 (3.2)	2897 (3.0)	3069 (3.0)	3454 (3.1)
Enterobacter spp.	1874 (2.5)	2256 (2.4)	2284 (2.4)	2314 (2.3)	2410 (2.2)
Proteus spp.	1662 (2.2)	1882 (2.0)	1818 (1.9)	1805 (1.8)	1839 (1.7)
Candida spp.	1042 (1.4)	1272 (1.4)	1376 (1.4)	1573 (1.5)	1687 (1.5)

Conclusions: The increase in blood culture reports is thought to be due to an increased number of rapid investigations of pyrexia of unknown origin (PUO) in patients presenting in the Accident & Emergency Department. Whilst the numbers of *E. coli* bacteraemia reports have increased, their proportion of all BSIs has remained stable. It is therefore unclear how many of the increased number of reports represent a true increase in case numbers and how many represent an increase in case reporting. The apparent increase in the proportions of coagulase-negative staphylococci requires further investigation. It is interesting to observe that no apparent increase in the proportion of candidaemia BSI was seen as has been reported in other countries where there are an increasing proportion of elderly and immunocompromised patients.

P680 In vitro activity of tigecycline tested against pneumonia pathogens from patients hospitalised in European medical centres, including multidrug-resistant *Acinetobacter* spp.

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Objectives: Emergence of resistance (R) among pneumonia-producing pathogens has been a variable that confounds empiric management. We evaluated the activity of tigecycline against leading bacterial pathogens recovered from patients hospitalised with pneumonia. Tigecycline was approved by the European Medicines Agency and US-FDA for the treatment of complicated skin and skin structure infections and intra-abdominal infections and is currently under investigation for treatment of hospital acquired pneumonia.

Methods: Consecutive, non-duplicate, lower respiratory tract isolates (3,864) were submitted from 34 medical centres located in Europe (13 countries) and Israel in the 2000–2007 period. Susceptibility (S) tests were performed using CLSI methods (including ESBL confirmatory tests) and interpreted by US-FDA and EUCAST criteria.

Results: Ranking of the top-10 occurring pneumonia pathogens and key resistance (R) characteristics were (see Table): *S. aureus* (28.3% oxacillin-R [MRSA]) > *P. aeruginosa* (19.6 imipenem [IMI]-R) > *E. coli* (8.5% ESBL) > *Klebsiella* spp. (28.9% ESBL) > *Enterobacter* spp. (25.6% ceftazidime [CAZ]-R) > *Acinetobacter* spp. > *S. pneumoniae* (23.1% penicillin-R) > *Serratia* spp. > *S. maltophilia* > *H. influenzae* (12.8% β-lactamase positive). *Acinetobacter* spp. exhibited high R rates to IMI (43.1%), CAZ (77.0%), ciprofloxacin (CIP; 82.8%) and amikacin (AMK; 65.1%); while 34.9% of strains were R to all 4 drugs (MDR). R to these antimicrobials did not adversely affect tigecycline activity, which inhibited >95% of strains non-S to CAZ, CIP or AMK, 93.3% of strains non-S to IMI and 91.8% of MDR strains at ⩽2 mg/L. Tigecycline was also very active against *S. maltophilia* (MIC90, 2 mg/L; 99.2% inhibited at ⩽2 mg/L), MRSA (MIC90, 0.25 mg/L; 99.5% S) and Enterobacteriaceae with an ESBL-phenotype, but showed limited activity against PSA.

Conclusions: Tigecycline is a potent agent targeting pneumonia pathogens displaying highly resistant phenotypes including *S. aureus*, Enterobacteriaceae, *S. pneumoniae*, *H. influenzae*, and some non-fermentative Gram-negative bacilli. Only tigecycline and the polymyxins showed reasonable in vitro activity against *Acinetobacter* spp. Empiric use of tigecycline may be prudent for patients less likely to have *P. aeruginosa* as a causative pathogen or for directed therapy of indicated species.

Organism (no. tested/% of total)	Tigecycline MIC (mg/L)			% S
	50%	90%	Range	(US-FDA/ EUCAST)
S. aureus (1,067/27.6)	⩽0.12	0.5	⩽0.12–1	99.8/99.8
P. aeruginosa (786/20.3)	>4	>4	⩽0.12–>4	–/–
E. coli (366/9.5)	⩽0.12	0.25	⩽0.12–1	100.0/100.0
Klebsiella spp. (353/9.1)	0.25	1	⩽0.12–4	98.6/92.4
Enterobacter spp. (223/5.8)	0.5	1	0.06–4	99.1/96.0
Acinetobacter spp. (209/5.4)	1	2	⩽0.12–4	97.1/77.5[a]
S. pneumoniae (121/3.1)	⩽0.12	⩽0.12	⩽0 12	–/–
Serratia spp. (121/3.1)	1	2	⩽0.12–4	99.2/92.6
S. maltophilia (114/3.0)	1	2	⩽0.12–4	99.2/77.2[a]
H. influenzae (109/2.8)	0.5	1	⩽0.12–1	–/–

a. Enterobacteriaceae breakpoints were used for comparison purposes only.

P681 In vitro activity of tigecycline against inpatient and outpatient isolates from a European population

M. Hackel, S. Bouchillon, B. Johnson, R. Badal, J. Johnson, D. Hoban, M. Dowzicky (Schaumburg, Collegeville, US)

Objectives: Tigecycline, a member of a new class of antimicrobials (glycylcyclines), has been shown to have potent broad spectrum activity against most commonly encountered species responsible for community and hospital acquired infections. The T.E.S.T. Program surveyed the in vitro activity of tigecycline and comparator compounds against in-patient and out-patient pathogens from Europe for the period from 2004 to 2007.

Methods: A total of 16,451 clinical isolates from 93 testing sites in 22 European countries were tested. Minimum Inhibitory Concentration (MICs) were determined by each site using common broth microdilution panels and interpreted according to EUCAST guidelines.

Results: See the tables.

	Enterobacteriaceae				*Acinetobacter* spp.			
	In-patients (n = 5,546)		Out-patients (n = 1,097)		In-patients (n = 926)		Out-patients (n = 148)	
	%S	MIC_{90}	%S	MIC_{90}	%S	MIC_{90}	%S	MIC_{90}
Tigecycline	91.7	1	95	1	na	1	na	1
Amikacin	95.9	4	96.5	4	72.7	>64	73.3	>64
Cefepime	82.3	8	85.8	4	59.8	32	60.3	>32
Ceftazidime	0	>32	0	32	57.4	>32	60.3	>32
Imipenem	98.9	1	98.8	1	82.5	16	78.4	8
Levofloxacin	82.2	8	83.4	8	55.2	>8	62.6	>8
Minocycline	82.9	8	86.7	8	92.4	4	98.5	1
Pip-Tazo	83.8	64	88.2	32	64	>128	70.2	>128

na = Breakpoints not available.

	S. aureus				*Enterococcus* spp.			
	In-patients (n = 1,415)		Out-patients (n = 374)		In-patients (n = 1,042)		Out-patients (n = 119)	
	%S	MIC_{90}	%S	MIC_{90}	%S	MIC_{90}	%S	MIC_{90}
Tigecycline	100	0.25	100	0.25	100	0.12	100	0.12
Levofloxacin	72.6	16	83.9	8	54.1	>32	69.3	>32
Linezolid	100	4	100	4	100	2	100	2
Minocycline	99	0.5	99	0.5	53.2	>8	41.6	>8
Vancomycin	100	1	100	1	93.9	2	95	2

Conclusion: Tigecycline's in vitro activity was comparable to or greater than most commonly prescribed broad spectrum antimicrobials without any demonstrable change in activity between in-patient and

out-patient bacterial study strains. Tigecycline's inhibitory activity against Enterobacteriaceae, and *Acinetobacter* spp. was comparable to imipenem. Against Gram-positive organisms, tigecycline's activity was comparable to linezolid and vancomycin.

P682 Comparative analysis of tigecycline in South America from 2004–2007

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Objectives: Tigecycline, the first member of the glycylcyclines, was first marketed in 2005 and has demonstrated success against multiple-resistant species and phenotypes. The T.E.S.T. Program is an ongoing global surveillance with the first post-marketing prospective report of tigecycline and comparator in vitro activity for the years 2004 through 2007.
Methods: 4,230 clinical isolates were collected from 16 investigative sites in 5 countries in South America. MICs were determined by broth microdilution according to CLSI guidelines using identical panels.
Results: Results are given by year for pathogens and antimicrobials. Summary data for tigecycline and key species are listed in the table.

Organism	N(04/05/06/07)	2004		2005		2006		2007	
		MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
Enterobacteriaceae	234/585/512/260	0.5	1	0.5	1	0.5	1	0.5	1
ESBL producers[a]	25/103/73/26	0.5	2	0.5	2	0.5	1	0.5	1
Acinetobacter spp.	53/111/59/50	0.5	1	0.5	1	0.5	1	0.5	1
P. aeruginosa	55/130/144/62	8	>16	8	16	8	>16	8	>16
S. aureus	64/215/116/97	0.06	0.12	0.12	0.25	0.12	0.25	0.12	0.25
MRSA	28/94/62/51	0.12	0.25	0.12	0.25	0.12	0.25	0.12	0.25
Enterococcus spp.	37/93/88/42	0.06	0.12	0.12	0.25	0.06	0.12	0.06	0.12
S. pneumoniae	41/96/57/52	0.25	0.5	0.12	0.5	0.015	0.06	0.015	0.06
H. influenzae	29/107/20/23	0.5	0.5	0.25	0.5	0.12	0.25	0.12	0.25

Conclusions: Other than a 1-doubling dilution increase in the MIC90 of *S. aureus*, tigecycline demonstrated no increase in MIC50/90 values over four years from its pre-marketing baseline values. Tigecycline's activity was retained even against strains resistant to other antimicrobials, including ESBL-producers, *Acinetobacter* spp., meticillin-resistant *S. aureus*, vancomycin-resistant enterococci, and penicillin-resistant *S. pneumoniae*.

P683 Tigecycline activity in Europe: a comparative analysis by country 2007

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Objectives: Development of bacterial resistance continues to cause concern world wide, but the availability of newer agents offers clinicians options for therapy. Tigecycline has a very broad spectrum of activity, including strains resistant to other drugs. As part of the global Tigecycline Evaluation Surveillance Trial (TEST), strains collected in 22 European countries in 2007 were evaluated for susceptibility to several commonly used antimicrobials.
Methods: 14,254 strains were collected and identified at 93 sites in 22 countries. MICs were determined at each site using EUCAST guidelines on microdilution panels.
Results: The table summarizes tigecycline results.
Conclusions: Bacteria isolated from than 22 different European countries had generally similar antibiograms, with no isolates from any site showing significantly different sensitivity patterns. The greatest variability was seen in *Acinetobacter*, with TIG MIC50 values ranging from 0.06 to 1 μg/mL. TIG's consistant broad spectrum of activity may make it an excellent therapeutic option against European pathogens.

Country	Enterobacteriaceae			*Acinetobacter* spp.			*S. aureus*			*Enterococcus* spp.		
	TIG		n	TIG		n	TIG		n	TIG		n
	MIC_{50}	MIC_{90}		MIC_{50}	MIC_{90}		MIC_{50}	MIC_{90}		MIC_{50}	MIC_{90}	
Austria	0.5	2	153	0.12	1	6	0.12	0.25	50	0.06	0.12	18
Belgium	0.5	2	645	0.25	1	118	0.12	0.25	208	0.12	0.25	131
Czech Republic	0.5	1	76	0.12	0.5	13	0.12	0.12	19	0.12	0.12	16
Denmark	0.5	1	283	0.12	0.25	49	0.12	0.12	76	0.06	0.06	57
Finland	0.25	0.5	75	0.25	1	9	0.12	0.12	31	0.12	0.25	17
France	0.5	1	1412	0.25	1	279	0.12	0.25	454	0.12	0.25	279
Germany	0.5	1	1174	0.12	0.5	214	0.12	0.25	345	0.12	0.25	219
Greece	0.5	1	372	0.5	2	70	0.12	0.25	127	0.06	0.12	77
Hungary	0.5	1	150	1	2	44	0.12	0.12	50	0.06	0.25	30
Ireland	0.5	2	222	0.12	0.5	45	0.12	0.12	74	0.06	0.25	42
Italy	0.5	1	1643	0.5	1	287	0.12	0.25	489	0.12	0.25	309
Latvia	0.5	1	145	0.5	1	29	0.12	0.25	49	0.06	0.12	30
Lithuania	0.25	1	66	0.25	1	15	0.12	0.12	35	0.12	0.25	15
Norway	0.5	0.5	71	0.06	0.25	15	0.12	0.12	24	0.06	0.12	15
Poland	0.5	1	75	0.25	0.5	14	0.12	0.12	24	0.12	0.12	15
Portugal	0.25	1	73	0.5	0.25	15	0.12	0.12	25	0.06	0.12	15
Slovenia	0.5	2	73	0.25	1	15	0.12	0.12	25	0.12	0.12	15
Spain	0.25	1	809	0.5	1	140	0.12	0.25	271	0.12	0.25	159
Sweden	0.25	0.5	209	0.12	0.12	44	0.12	0.12	57	0.06	0.12	45
Switzerland	0.5	0.5	157	0.12	0.25	20	0.12	0.12	51	0.12	0.12	33
The Netherlands	0.25	0.5	149	0.12	0.5	30	0.12	0.12	49	0.12	0.12	30
United Kingdom	0.25	1	361	0.12	1	72	0.12	0.25	120	0.12	0.12	98

P684 In vitro activity of tigecycline and 13 comparators against pathogens from a global population of intensive care patients in 2007

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Objectives: Tigecycline is a novel antimicrobial from a new class of compounds, the glycylcyclines, which has been shown to have potent broad spectrum activity against most commonly encountered species responsible for community and hospital acquired infections. The Tigecycline Evaluation Surveillance Trial (TEST), a global longitudinal surveillance study, surveyed the in vitro activity of tigecycline and 13 comparator compounds against pathogens from ICU patients from a global population during 2007.
Methods: A total of 14,113 clinical isolates from 386 testing sites in 49 countries were evaluated. Minimum Inhibitory Concentration (MICs) were determined by each site using common broth microdilution panels and interpreted according to CLSI and FDA guidelines.
Results: See the tables.

Drug	Enterobacteriaceae (n = 7,576)		*Acinetobacter* spp. (n = 1,646)	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
Tigecycline	0.5	2	0.5	1
Amikacin	2	4	8	>64
Cefepime	⩽0.5	8	16	>32
Imipenem	0.5	1	1	>16
Levofloxacin	0.06	8	4	>8
Minocycline	2	16	⩽0.5	8
PipTazo	2	128	64	>128

Drug	*S. aureus* (n = 1,722)		*Enterococcus* spp. (n = 1,252)	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
Tigecycline	0.12	0.25	0.06	0.12
Levofloxacin	0.25	32	16	>32
Linezolid	2	2	2	2
Minocycline	⩽0.25	0.5	4	>8
Vancomycin	1	1	1	>32

Conclusion: Tigecycline's in vitro activity was comparable to or greater than most commonly prescribed broad spectrum antimicrobials for all ICU bacterial study strains encountered. Tigecycline's inhibitory activity against Enterobacteriaceae, and *Acinetobacter* spp. was comparable to imipenem. Against Gram-positive organisms, tigecycline's MIC90 values of ⩽0.25 μg/mL suggest that tigecycline may be an effective and reliable therapeutic option against these pathogens in an Intensive Care setting.

P685 Tigecycline activity tested against bacterial isolates causing bloodstream infections in European medical centres

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Objective: To assess the activity of tigecycline against bloodstream infection (BSI) isolates from European hospitals. Tigecycline is a novel glycylcycline antimicrobial approved by the European Medicines Agency for the treatment of complicated skin and skin structure infections and intra-abdominal infections.

Methods: Bacterial isolates (non-duplicates) were consecutively collected in the 2000–2007 period from documented BSI in patients hospitalised in 34 medical centres located in Europe (13 countries) and Israel. Frequency of occurrence of pathogens was determined and their antibiograms assessed using reference broth microdilution methods according to CLSI M7-A7 (2006). Tigecycline-susceptible (S) breakpoints (US-FDA/EUCAST) were defined as ⩽2/⩽1 mg/L for Enterobacteriaceae (ENT); ⩽0.5/⩽0.5 mg/L for staphylococci, and ⩽0.25/⩽0.25 mg/L for streptococci and enterococci.

Results: A total of 25,401 strains were tested. Tigecycline was highly active against the 10 most frequent pathogens (Table), except for *P. aeruginosa* (PSA). Among the 5 most common pathogens (18,802 strains; 74% of the total), tigecycline was active against >99% at the established S breakpoints. The main resistance phenotypes detected were meticillin-resistant (R) *S. aureus* (MRSA; 28.3%) and CoNS (77.6%), ciprofloxacin-R *E. coli* (20.3%), extended-spectrum β-lactamase (ESBL)-screen-positive *Klebsiella* spp. (22.8%) and *E. coli* (7.9%), imipenem-R PSA (IRPSA; 21.7%) and *Acinetobacter* spp. (ASP; 30.5%), and vancomycin-R enterococci (VRE; 5.0%). Tigecycline activity against MRSA (MIC90, 0.25 mg/L; 99.8% S) was similar to that against meticillin-S *S. aureus* (MIC90, 0.25 mg/L; 99.9% S), and 98.0% of VRE were S to tigecycline. ESBL-producing *E. coli* and KSP exhibited high rates of R to levofloxacin (62.4 and 31.8%, respectively) and gentamicin (31.7 and 45.1%), but were S to tigecycline (100.0 and 98.9%, by US-FDA breakpoints). 95.3% of ceftazidime-R ESP and 96.1% of imipenem-R ASP were inhibited at ⩽ 2 mg/L of tigecycline.

Organism (no. tested/% of total)	Cumulative % inhibited at tigecycline MIC (mg/L) of:						% S
	⩽0.12	0.25	0.5	1	2	4	(US-FDA/ EUCAST)
E. coli (5,793/22.8)	66.5	95.7	99.6	99.9	>99.9	100.0	>99.9/99.9
S. aureus (5,642/22.6)	68.7	95.3	99.9	100.0	–	–	99.9/99.9
Coagulase-neg. staphylococci (CoNS; 3,396/13.4)	57.0	88.8	98.9	100.0	–	–	98.8/98.9
Enterococcus spp. (2,265/8.9)	75.3	97.0	99.9	100.0	–	–	97.0/97.0
Klebsiella spp. (KSP; 1,706/7.6)	8.5	58.7	87.3	96.2	99.4	>99.9	99.4/96.2
P. aeruginosa (PSA; 1,481/5.8)	0.1	0.3	1.1	2.4	11.1	45.9	–/–
Entertobacter spp. (ESP; 977/3.8)	2.3	44.8	81.9	92.2	97.9	100.0	97.9/92.2
S. pneumoniae (551/2.2)	99.5	100.0	–	–	–	–	100.0/100.0
Beta-haemolytic streptococci (535/2.1)	98.5	100.0	–	–	–	–	100.0/100.0
Acinetobacter spp. (ASP; 509/2.0)	18.7	39.3	55.8	83.5	97.6	100.0	97.6/83.5[a]

[a] ENT breakpoints were applied for comparison purposes.

Conclusions: Tigecycline exhibited a wide-spectrum of activity and potency versus contemporary BSI isolates collected in Europe, including multidrug-resistant organisms.

P686 Extended-spectrum β-lactamase production in Europe

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Objectives: Infection by extended-spectrum beta lactamase (ESBL)-producing bacterial pathogens is increasing worldwide, with the prevalence of ESBLs in Europe varying greatly from country to country. Patients with infections caused by an ESBL-producing organism are at increased risk of treatment failure. The Tigecycline Evaluation Surveillance Trial (TEST), a global longitudinal surveillance study, determined the ESBL status and antibiotic susceptibilities to tigecycline and comparator compounds for 6,782 *Escherichia coli*, *Klebsiella oxytoca*, and *Klebsiella pneumoniae*, from 22 European countries from 2004 to 2007.

Methods: 6,782 Enterobacteriaceae (2,871 *E. coli*, 815 *K. oxytoca*, 1,938 *K. pneumoniae*) from 22 European countries were analysed in this survey. The isolates were identified to the species level at the participating sites and confirmed by the central laboratory. MICs were determined by each site using supplied broth microdilution panels and interpreted according to EUCAST guidelines. ESBL testing was performed by Laboratories International for Microbiology Studies (LIMS), a subsidiary of International Health Management Associates, Inc. (IHMA, Schaumburg, IL, USA) following CLSI guidelines.

Results: See the table.

	2004		2005		2006		2007	
	n (% ESBL)	TIG MIC_{90}	n (% ESBL)	TIG MIC_{90}	n (% ESBL)	TIG MIC_{90}	n (% ESBL)	TIG MIC_{90}
E. coli	558 (3.9)	0.12	525 (9.7)	0.12	994 (8.7)	0.12	794 (9.8)	0.25
ESBL	22	0.25	51	0.25	86	0.25	78	0.25
K. oxytoca	148 (5.4)	0.25	142 (2.8)	0.25	272 (2.9)	0.25	253 (2.8)	0.5
ESBL	8	0.5	4	0.5	8	0.25	7	0.5
K. pneumoniae	393 (13.0)	0.5	305 (14.4)	0.5	724 (9.7)	0.5	516 (15.5)	0.5
ESBL	51	0.5	44	0.5	70	0.5	80	0.5
Total	1099 (7.4)	0.25	972 (10.2)	0.25	3148 (5.2)	0.25	1563 (10.6)	0.25
ESBL	81	0.5	99	0.5	164	0.25	165	0.5

Conclusions: Rates of ESBL production in Europe have increased gradually during the period of 2004 to 2007, with a decrease in the year 2006. The incidence of ESBLs varies greatly from country to country, ranging from 2.6% in Denmark to 24.2% in Greece in 2007 (data not shown). Tigecycline retained excellent in vitro activity over time, with MIC90 values of ⩽0.5 μg/mL for all four years, suggesting tigecycline may be an effective option against these difficult to treat pathogens.

P687 The in vitro activity of tigecycline against 2,423 clinical isolates. Comparison of the EUCAST and the FDA approved breakpoints

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Objectives: The multicentre comparison of the in vitro activities of tigecycline and comparator agents against Gram-negative and Gram-positive clinical isolates.

Materials and Methods: During 2006–2007, a total of 2,423 non-duplicate clinical isolates were collected from 17 General Hospitals in Greece. MICs to tigecycline, penicillin, ampicillin, amoxicillin+clavulanic acid, piperacillin+tazobactam, cefotaxime, ceftazidime, cefepime, imipenem, amikacin, minocycline, levofloxacin, vancomycin, and linezolid were determined using a microdilution method.

Results: Species distribution included *Escherichia coli* (464, 18.8%), *Klebsiella pneumoniae* (350, 14.2%) *Enterobacter* spp. (247, 10.0%), *Acinetobacter* spp. (235, 9.5%), *Moraxella catarrhalis* (39, 1.6%), *Enterococcus* spp. (513, 20.8%), *Staphylococcus aureus* (505, 20.1%) and *Streptococcus pneumoniae* (70, 2.8%). Isolates were collected from skin and soft tissue, blood, urine, respiratory tract, intra-abdominal, iv catheter, biological fluids, and genital specimens (20.6, 18.8, 18.6, 15.2, 10.8, 3.7, 3.3, and 1.9%, respectively). Tigecycline MIC50, MIC90, as well as susceptibility rates according to FDA and EUCAST approved genus/species specific breakpoints, are shown in Table.

No significant differences were recorded between meticillin-resistant and susceptible *S. aureus*, vancomycin-resistant and susceptible *Enterococcus* spp., penicillin-resistant and susceptible *S. pneumoniae*, or imipenem-resistant and susceptible *Acinetobacter* spp.

Genus	N	MIC (mg/L)		FDA breakpoints**			EUCAST breakpoints**		
		50%	90%	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
E. coli	464	0.12	0.5	99	0.5	0.5	99	0	1
K. pneumoniae	350	0.5	1	99	0.5	0.5	91	8	1
Enterobacter spp.	247	0.5	2	93	5	2	88	6	6
ImpNS* *Enterobacteriaceae*	110	0.5	1	99	0	1	94	5	1
Acinetobacter spp.	234	1	1	No breakpoints available			No breakpoints available		
M. catarrhalis	40	0.03	0.12	No breakpoints available			No breakpoints available		
Enterococcus spp.	513	0.12	0.12	100	0	0	99	1	0
S. aureus	505	0.12	0.25	100	0	0	99.5	0	0.5
S. pneumoniae	70	0.5	1	No breakpoints available			No breakpoints available		

*ImpNS: Imipenem Non-Susceptible; **S: susceptible, I: intermediate, R: resistant.

Conclusions: Tigecycline exhibited potent in vitro activity against the majority of the clinical isolates in this study. Species with the highest MICs recorded were *S. pneumoniae* among Gram-positive cocci and *Enterobacter* and *Acinetobacter* spp. among Gram-negative bacteria. Interpretation of the results, using the different breakpoints established by the two organisations, may influence substantially the resistance rates reported for *K. pneumoniae* and *Enterobacter* spp.

P688 Analysis of resistance and vancomycin "reverse creep" in Latin American *Staphylococcus aureus*: ten-year report of the SENTRY Antimicrobial Surveillance Program (1997–2006)

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Objectives: To evaluate the antimicrobial susceptibility (S) rates of *S. aureus*, including meticillin-resistant (MRSA) strains, collected in Latin American medical centres as part of the SENTRY Antimicrobial Surveillance Program. The Brazilian MRSA clone is highly prevalent in the region and usually shows high rates of antimicrobial resistance.

Methods: A total of 8,705 *S. aureus* referred from seven Latin American countries during a 10-year period (1997–2006) were S tested to 13 antimicrobials by CLSI broth microdilution methods. The frequencies of *S. aureus* with vancomycin MIC values ⩾1 mg/L collected by 7 of 10 medical centres that participated in the programme since 1999 were analysed by comparing two time periods, 1999–2001 and 2002–2006.

Results: Overall, 37.3% of strains were MRSA. Brazil contributed with the majority of *S. aureus* (3517/40.4%) and MRSA (1443/41.1%) strains. S rates are shown in the table. MRSA rates increased from 33.8% in 1997 to 40.2% in 2006 ($p = 0.007$). S rates for many non-β-lactam agents increased among MRSA as follows (1997/2006): clindamycin (13.2/16.3%), erythromycin (3.9/8.6%), gentamicin (7.8/27.3%), tetracycline (29.9/79.5%), co-trimoxazole (30.9/67.0%). Chloramphenicol and rifampin S rates increased from 25.5% and 48.5% in 1997 to 65.6% and 60.7% in 2005, respectively. All increases in S rates among MRSA were statistically significant ($p < 0.05$), except for clindamycin. Interestingly, the percentage of *S. aureus*/MRSA with vancomycin MIC ⩾1 mg/L decreased from 93.9/96.6% in 1999–2001 to 86.7/92.3% in 2002–2006 ($p = 0.00001$).

Antimicrobial agent	MRSA (n = 3253)			MSSA (n = 5452)		
	MIC (mg/L)		% S	MIC (mg/L)		% S
	50%	90%		50%	90%	
Ciprofloxacin	>4	>4	8.3	0.25	0.5	96.2
Clindamycin	>8	>8	12.4	0.12	0.25	98.0
Gentamicin	>8	>8	15.6	⩽2	⩽2	95.9
Erythromycin	>8	>8	6.9	0.25	8	82.1
Linezolid	2	2	100.0	2	2	100.0
Teicoplanin	⩽2	2	100.0	⩽2	⩽2	100.0
Co-trimoxazole	>2	>2	65.1	⩽0.5	⩽0.5	98.8
Vancomycin	1	1	100.0	1	1	100.0

Conclusions: The increase in the MRSA rates coupled with increased S rates to non-β-lactam agents and decrease in the frequencies of strains with vancomycin MIC ⩾1 mg/L ("reverse creep") may indicate the emergence and dissemination of new MRSA clones, distinct from the Brazilian clone, in the Latin American countries surveyed by the SENTRY Program.

P689 Frequency and antimicrobial susceptibility profile of vancomycin-resistant enterococci from Latin America: a report from the SENTRY Antimicrobial Surveillance Program (1997–2006)

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Objective: To evaluate the prevalence and susceptibility profile of vancomycin-resistant enterococci (VRE) in Latin America. VRE emerged nearly twenty years ago in the United States (USA) and rapidly disseminated in many USA hospitals; however, its prevalence seems to remain relatively low in Latin America.

Methods: As part of the SENTRY Antimicrobial Surveillance Program, a total of 2026 enterococci isolated between January 1997 and December 2006 from Latin American hospitals were studied. The susceptibility profile to nine antimicrobial agents was determined by CLSI broth microdilution methods and results interpreted according to CLSI document M100-S17 (2007).

Results: *E. faecalis* (78.2%) and *E. faecium* (12.7%) were the most frequently isolated species. Blood (41.4%) and skin/soft tissue (11.0%) represented the most frequent body sites of infection. A total of 112 (5.5%) strains were identified as VRE, with 46% of them being isolated in Brazil. The VRE frequency increased from 0.0% (1997 and 1998) to 10.3% (2006), with an important increase after 2002. Although the numbers of *E. faecalis* and *E. faecium* exhibiting the VRE phenotype were similar, the percentage of vancomycin-resistant *E. faecium* (VR-EFM; 19.7%) was proportionally higher than vancomycin-resistant *E. faecalis* (VR-EF; 3.3%). A significant increase in VR-EFM isolates was detected in Brazil between 2005 and 2006. VRE was most frequently isolated from urine (18.5%). The antimicrobial susceptibility profile of VRE isolates is summarised in the table. Linezolid and tigecycline were the most active compounds tested against VRE. In contrast, the majority of VRE were resistant to teicoplanin (vanA pattern).

Antimicrobial agent	VR-EF (53)			VR-EFM (51)		
	MIC_{50}	MIC_{90}	%S	MIC_{50}	MIC_{90}	%S
Ampicillin	4	16	86.8	>16	>16	2.0
Teicoplanin	>16	>16	3.8	>16	>16	5.9
Gentamicin-HL	>1000	>1000	18.9	⩽500	>1000	76.5
Streptomycin-HL	⩽1000	>2000	83.0	>2000	>2000	15.7
Quinupristin/dalfopristin	>2	>8	1.9	1	>2	68.6
Linezolid	1	2	100.0	1	2	100.0

Conclusions: An important increase in the frequency of VRE has been observed in Latin America and appears to be associated with the dissemination of endemic clones expressing the vanA. Linezolid and tigecycline were the only antimicrobials with broad activity against *Enterococcus* spp. independent of the species identification or glycopeptide phenotype.

P690 Prevalence of vancomycin-resistant *Enterococcus* spp. and associated resistance patterns among isolates collected over a decade of SENTRY Program surveillance in North America

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Objective: To investigate the susceptibility pattern of *E. faecalis* and *E. faecium* in the USA and Canada medical centres over 10 years;

secondarily, to analyse trends in vancomycin-resistant *Enterococcus* spp. (VRE) in North America during the same period.
Methods: Isolates from bloodstream infection (BSI; 7,284) and other sources (2,763; mainly urine and wound) recovered as part of a 10-year surveillance programme (1997–2006) were analysed for susceptibility profiles. Isolates were collected from medical centres in the USA (52) and in Canada (8) and were tested at a central laboratory (JMI Laboratories, North Liberty, IA) using CLSI broth microdilution methods and interpretation criteria.
Results: Overall, the VRE rate was higher in BSI (19.1%) compared to other sources (14.5%). In the USA, VR *E. faecalis* rates were stable over ten years (3.3%) with significant year-to-year variation but without trending. In contrast, VR *E. faecium* steadily increased from 50 to 70%. The percentage of vanA phenotype increased in both species over the years, becoming the predominant resistance phenotype in more than 85% of VRE isolates. The table shows the yearly progression of VRE BSI isolates in the USA and van phenotypes. In Canada, VRE was documented in <1% of BSI isolates and included *E. faecium* only. Higher resistance rates to other agents were noted among VRE compared to susceptible strains. These included ampicillin-resistant *E. faecalis* at 13%, ciprofloxacin-resistance at ⩾95% for both species and high-level gentamicin and streptomycin resistance rates were 20–40% higher among VRE compared to vancomycin-susceptible strains. Linezolid-resistance was 1.3% and 2.5% among VR *E. faecium* and *E. faecalis*, respectively, compared to <0.5% among vancomycin-susceptible isolates.
Conclusions: At the beginning of this study (1997), VRE were considered to be primarily a USA problem. As expected, VRE has disseminated within other countries, becoming a worldwide concern. The spread of VRE in North America warrants continued susceptibility profiling of large numbers of isolates from multiple medical centres to track changes. The 20% *E. faecium* VR increase in the USA (nearly all caused by the presence of vanA) and an associated increase in co-resistance has been alarming. Other countries should take note of the USA VRE experience and initiate infection control measures to limit this important pathogen from becoming widely endemic.

	Year									
Organism	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006
E. faecalis										
n	371	378	408	348	361	487	431	466	415	436
%R[a]	4.9	2.9	0.7	4.0	1.4	2.9	4.6	2.6	4.3	5.0
%vanA	55.6	45.5	66.7	42.9	40.0	57.1	35.0	50.0	50.0	86.4
E. faecium										
n	130	144	136	133	155	167	190	231	224	281
%R[a]	50.0	50.0	61.0	57.1	60.0	70.7	70.5	68.0	70.5	69.4
%vanA	75.4	88.9	80.7	84.2	88.2	86.4	88.1	94.9	95.6	99.0

[a]Represents vancomycin non-susceptible rates (⩾8 μg/ml, CLSI M100-S17).

Biofilms

P691 Effect of fluoride content of the oral biofilm on the surface structure of titanium

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Background: High fluoride concentration and the low pH in prophylactic mouthwash and gels used to prevent caries may modify the surface structure of the transgingival region of implants made of titanium (Ti). Fluoride, like a reductive agent may have an opposite effect and attack the oxide layer. The aim of the present work was to study whether the changes on the surface structure of Ti caused by high fluoride concentration and acidic pH alter the adherence and colonisation of bacteria.
Methods: Polished commercially pure titanium CP grade 4 discs (Camlog) were used. Each sample was treated for 1 hour with one of the solutions: mouthwash containing 0.025% fluoride, a gel containing 1.25% fluoride and a solution of 1.0% NaF (pH 4.5). The surface structures were analysed by atomic force microscopy (AFM) and X-ray photoelectron spectroscopy (XPS). The colonisation of *Porphyromonas gingivalis* strains was studied by scanning electron microscope (SEM) after 5 days of anaerobic incubation and the quantity of the bacterial protein was determined by commercially available kit (Pierce).
Results: The roughness of the treated sample surfaces (Ra), as revealed by the AFM measurements, was increased significantly (2 times for the gel and 10 times for the 1% NaF solution) as compared to the control surface. No changes were detected in case of the mouthwash. The high fluoride concentration and acidic pH in the case of the gel and the 1% NaF solution resulted in a strong corrosion and a modification of the composition of the titanium surface. The XPS spectra revealed formation of fluoride containing complexes (NaF and Na2TiF6) bound strongly to the surface. A correlation was revealed between the roughness of the surface and the thickness and the maturity of the *P. gingivalis* bacterial colonies developed on the titanium surface. The number of the bacteria was significantly increased due to the changes in the surface caused by NaF treatment.
Conclusions: High fluoride concentration and acidic pH increase the roughness of the titanium surface. Bacterial biofilm colonisation on this rough surface was proved to be more mature. The present study indicates that high fluoride concentration and acidic pH may affect the development of transgingival epithel junction on the Ti surface.
This work was supported by the GVOP-3.2.1.-2004–04–0408/3.0 project funded by the EC and the Hungarian Ministry of Economy, and the ETT project (434/2006) supported by the Hungarian Ministry of Health.

P692 Are silver-coated Foley catheters resistant to encrustation by crystalline bacterial biofilms?

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Objectives: To examine (1) the vulnerability of silver-coated silicone and silver/hydrogel-coated latex catheters to colonisation by crystalline *Proteus mirabilis* biofilm; (2) to determine the extent to which silver ions elute into urine from these catheters.
Methods: Laboratory models of the bladder were fitted with size 14 Rüsch silver-coated silicone catheters or Bard IC silver/hydrogel-coated latex catheters. Rüsch all-silicone catheters were used as non-coated controls. Models were supplied with artificial urine at 1 ml/min and inoculated with 10 ml of a 4 h urine culture (2 x 10^8 cfu/ml) of *P. mirabilis* NSM6, a test strain that had been isolated from the blocked catheter of a patient. Models were operated until the catheters blocked. Scanning electron microscopy was used to visualize the biofilms that had developed on the catheters. The silver content of urine flowing from the catheters over three day periods from models that had not been inoculated was assayed by atomic absorption spectroscopy.
Results: The mean times the models took to block calculated from data obtained from five replicated experiments were 54.3±15.8h for the silver/hydrogel-coated catheters, 73.5±23.0h for the silver/silicone devices and 65.0 ±11.3h for the controls. Analysis of variance revealed no significant differences between these mean values (P = 0.257). Scanning electron microscopy confirmed that catheter blockage was due to crystalline biofilm formation. The eye-hole and lumenal surfaces of all three types of catheter were shown to be colonised by extensive co-aggregates of cells and crystals. Analysis of urine failed to detect silver in the urine draining from the catheters (limit of detection 0.05 mg/L). At blockage the mean viable cell populations in the urine from the models were 1.8 x 10^7 cfu/ml for the control, 3.4 x 10^7 cfu/ml for the silver/silicone catheter and 2.9 x 10^7 cfu/ml for the silver/hydrogel catheter. One-way analysis of variance revealed no significant difference in these mean values (P = 0.148).
Conclusions: The silver-coated silicone and the silver/hydrogel-coated latex catheters are equally vulnerable to encrustation and blockage by crystalline bacterial biofilm. The concentrations of silver eluting into urine from these catheters are less than 0.05 mg/L and are not antibacterial.

P693 ***Staphylococcus aureus* biofilm eradication: a new approach by phodynamic therapy**

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Introduction: Biofilms have remarkable medical importance for several infections. Today bacterial biofilm properties are not fully understood and strategies for their treatment remain undeveloped. Standard antimicrobial treatments often fail in biofilm eradication and chronic infections can arise. The development of alternative therapies against biofilm infections represents one of the major public health concern. Recently, photodynamic therapy (PDT), is receiving considerable interest for its potential as antimicrobial therapy. The principle of PDT is rather simple: a photosensitizer is taken up into cells and activated by light of appropriate wavelength causing cell death through the production of active oxygen species (ROS).
Objectives: The aim of this study was to investigate the efficacy of cationic Zn(II)phthalocyanines (PC) of novel synthesis as photosensitizers for selective in-vitro eradication of *S. aureus* biofilm.
Methods: *S. aureus* ATCC 6538 biofilm was carried out in a 96-wells flat bottom sterile polystyrene microplates. After 24h incubation at 37ºC, appropriately diluted Zn(II)PC solutions (0.05–50 microM) were added to each well. After 5 min incubation in the dark Zn(II)PC-treated biofilms were irradiated with red light (30 J/cm^2). Biofilms residual vitality after photoinactivation was determined by XTT assay. Cell survival was expressed in terms of the percentage inhibition of XTT formazan production (measured at 450 nm) by treated biofilms compared with values obtained for untreated biofilms.
Results: The antibacterial kiling effects on the *S. aureus* biofilms, after photodynamic treatment with cationic Zn(II)PC, were linear in a concentration-dependent manner. The 50% cell killing was obtained at a very low concentration 0.05 microM with the most effective compound and at least 95% killing was caused by all the Zn(II)PC tested at higher concentration (50 microM).
Conclusion: Our results have shown that cationic Zn(II)PC photosensitizers in conjunction with red light (600–700 nm) of moderate intensity display a potent in vitro activity against sessile *S. aureus* cells. The photodynamic inactivation by Zn(II) PCs resulted more effective or rapid than vancomincin, one of the most common antibacterial agents used for the Sthaphylococcus spp. treatment, indicating their potential usefulness for the eradication of *S. aureus* biofilm-associated infection.

P694 **The possible role of the LPXTG protein SesC in *Staphylococcus epidermidis* biofilm formation**

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Objective: Several well-studied proteins with defined roles in *Staphylococcus epidermidis* biofilm formation (Aap, Bhp, SdrF and SdrG) are LPXTG motif containing proteins. We investigated the involvement of another LPXTG protein (SesC -Accession: NP_765787-Bowden et al. Microbiology. 2005; 151:1453) in biofilm formation.
Methods: Different clinical isolates of staphylococci were tested for the presence of sesC by PCR. Our previously described in vitro and in vivo rat models were used to investigate the time course of gene expression in planktonic and sessile bacteria during biofilm formation and persistence. A recombinant protein of SesC was produced and used to raise polyclonal antisera in rabbits. Immobilised recombinant SesC was used for polyclonal antibody purification from rabbit serum. ELISA and Western blot were performed to demonstrate the presence of SesC in the cell-wall fraction. Purified anti-SesC IgG's and FITC-labelled goat-anti-rabbit antibodies were used in immunofluorescence assays to study in vitro protein expression and the effect on biofilm formation. The sesC gene was cloned in pCN68 (AEM. 2004, 70:6076) and expressed in sesC negative strains of *S. aureus* RN4220 and a clinical isolate of *S. haemolyticus*. Biofilm formation ability of transformed strains was compared to that of wild strains.
Results: The sesC gene was present in all *S. epidermidis* strains. The expression of sesC was significantly upregulated during the early and late in vivo infection. A small statistically not significant difference in gene expression between sessile and planktonic bacteria was observed in vitro. Fluorescence microscopy data confirmed the gene expression study data. Furthermore, we demonstrated that polyclonal antibodies against SesC reduced in vitro *S. epidermidis* biofilm formation. Expression of sesC in non *S. epidermidis* strains didn't lead to any significant difference in biofilm formation ability of the transformed strains.
Conclusion: The presence of sesC in all tested *S. epidermidis* strains but not in all other staphylococcal species indicates the importance of sesC for *S. epidermidis*.
The observed upregulation in sessile bacteria and the ability of polyclonal anti-SesC antibodies to inhibit biofilm formation in *S. epidermidis* suggest a role for SesC in the *S. epidermidis* biofilm formation. The precise role in biofilm formation of this protein remains to be solved.

P695 **Detection of staphylococcal ability for biofilm formation in otitis media in children. An evaluation of three different detection methods**

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Objectives: Chronic otitis media (OM) is a common paediatric infectious disease. The infection has long been considered to be a sterile inflammatory process. The previous application of highly sensitive techniques of molecular biology suggests that metabolically active bacteria exist in culture-negative paediatric middle-ear effusions. To explain these observations it has been previously proposed that chronic OM may result from a mucosal biofilm infection. Bacterial biofilms are difficult to detect in routine diagnostics and are inherently tolerant to host defences and antibiotic therapies. In addition, biofilms facilitate the spread of antibiotic resistance by promoting horizontal gene transfer. The purpose of this study was to analyse a phenotypic and genotypic ability of isolated staphylococcal strains for biofilm formation during the course of otitis media in children by using three different detection methods.
Methods: A total of 96 effusions from the mid-ear, obtained from 48 patients aging between 2 and 6 with chronic otitis media, were tested bacteriologically by conventional methods. The identification to species level was performed by using API tests (Bio Merieux). Among 36 culture positive specimens, 16 strains of *Staphylococcus* spp. were screened for biofilm formation by tissue culture plate (TCP), Congo red agar (CRA) method and presence of ica A, B, C, D operons.
Results: Of 16 *Staphylococcus* spp., Api Staph revealed 8 strains of *S. epidermidis*, two S. sciuri, one *S. capitis*, one *S. caprae*, one *S. warneri*. The remaining three strains were identified as *S. aureus*.
Regarding coagulase negative Staphylococci (CNS) isolates, 6 (37.5%) revealed the phenotypic ability to produce biofilm, confirmed by TCP and CRA methods. Three of these isolates (18.7%) were also icaA and icaD positive and slime forming. Two *S. aureus* strains, detected as icaA or icaB positive by PCR analysis displayed a biofilm-negative phenotype in TCP and CRA methods.
Conclusion: Among the *Staphylococcus* genus, the species that is most commonly involved in biofilm formation is *S. epidermidis*. Biofilm formation is encoded by the ica operon and coexpression of icaA and icaD is required for full slime synthesis. TCP method correlates with the results of ica detection and is more specific than CRA.

P696 ***Staphylococcus epidermidis* biofilm modulates cytokine production**

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Objectives: To compare the immune response of *Staphylococcus epidermidis* bacterial cells existing either in planctonic or in biofilm phase.
Methods: A reference slime producing *S. epidermidis* strain (ATCC35983) was used in the present study. Slime production was

confirmed by Christensen's method. Planctonic phase bacteria were obtained after a 6h incubation in proper medium. Biofilm phase bacteria were obtained by incubation in 24-well tissue culture plates for 48h and subsequent rinse; biofilm phase cells remained attached to the bottom of the wells. Peripheral blood mononuclear cells from multiple healthy donors were isolated and co-cultured in 24-well flat bottom tissue culture plates either with live *S. epidermidis* planctonic cells at a ratio of 25:1 or with biofilm phase cells. Cytokines were measured in triplicate, using the Luminex® xMAP™ technology, in cell-free supernatants collected at 2, 4, 6, 12, 24 and 48 hours. The 8-plex assay kit was used for the simultaneous quantitative determination of the following cytokines: TNFa, IL-1b, IL-6, IL-8, IL-10, IL-12p40, IL-13 and GM-CSF, whereas, the 3-plex high-sensitivity assay kit was used for IL-12p70, IL-2, and INF-g.

Results: Kinetics of cytokine production revealed that TNFa and IL-10 accumulation was higher at 12h, whereas IL-12, IL-2, INF-g, and GM-CSF peaked at 48h. IL-1b, IL-6 and IL-8 levels exhibited a plateau pattern between 6 and 48h. IL-13 was not detectable in most experiments. Biofilm phase bacteria elicited production of lower levels of all cytokines tested as compared to planctonic phase bacteria. This phenomenon was more prominent for IL-12p40, as biofilm phase bacteria led to 35-fold lower IL-12p40 production. Although cytokines' levels showed interdonor variability, the overall pattern was reproducible in all experiments. Differences were statistically significant ($p < 0.05$). Mean peak value±standard deviation of each cytokine in pg/mL from a typical representative experiment is shown in Table 1.

	TNFa	IL-1b	IL-6	IL-8	IL-10	IL-12p40	IL-12p70	IL-2	INF-g	GM-CSF
Planctonic phase	5200 ±270	8500 ±460	9460 ±350	8900 ±550	212 ±30	120 ±18	1.79 ±0.3	3.04 ±0.26	5.7 ±1.5	130 ±14
Biofilm phase	210 ±25	749 ±60	420 ±35	2800 ±340	80 ±15	3.5 ±0.4	0.11 ±0.02	0.13 ±0.03	2.04 ±0.35	60 ±8

Conclusion: *S. epidermidis* cells embedded in biofilm induce low-level production of proinflammatory and adaptive immunity cytokines, especially IL-12p40, which is a key cytokine in development of TH1 responses leading to suppression of immune system, and may explain observed silent, persistent, infections.

P697 The coupled effects of physicochemical and flow conditions on *Staphylococcus epidermidis* adhesion and ica genes expression

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Objectives: Meticillin resistant *Staphylococcus epidermidis* (MRSE) is the predominant species associated with medical device infections impeding their long-term use. The critical step to development of infections is bacterial adhesion to biomaterials mediated by non specific interactions, as well as bacterial slime production encoded by the ica operon. The coupled effects of specific chemical terminal surface groups and flow conditions on the initial adhesion of *S. epidermidis* were investigated in correlation to the expression of two genes of the ica operon.

Methods: Reference control strains (ATCC35984, slime-positive and ATCC12228, slime-negative), and two clinical strains isolated from different hospitalised patients, (one ica-positive/slime-positive and one ica-positive/slime-negative) were investigated. Bacteria were grown in BHI medium and then suspended in physiological saline at a concentration of ~3×10^9 cells/mL. Hydroxyl (OH)-terminated (hydrophilic) and methyl (CH3)-terminated (hydrophobic) glass surfaces were used as substrates in a parallel flow chamber. Bacterial adhesion was examined under two flow rates: 2mL/min and 20mL/min for four hours. Total RNA from both bacterial supernatant (s) and adherent (a) bacteria after detachment with trypsin, was isolated by the Trizol method. Reverse transcription followed by quantitative Real-Time PCR (qRT-PCR) allowed the detection of expression levels of icaA and icaD. Table 1 summarizes the results.

Table 1: Quantification of *icaA* and *icaD* expression by qRT-PCR

Bacterial strain	Slime	qRT-PCR															
		icaA (copies/µL)								*icaD* (copies/µL)							
		(OH), ml/min				(CH_3), ml/min				(OH), ml/min				(CH_3), ml/min			
		2		20		2		20		2		20		2		20	
		s	a	s	a	s	a	s	a	s	a	s	a	s	a	s	a
ATCC 35984	+	0	0	8	0	0	0	13	5	80	52	583	56	104	114	1864	8
ATCC 12228	–	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2264	–	1	0	0	2	5	0	0	4	11319	108	36	2	161	2	121	3
4388	+	3	0	3	1	8	5	0	3	3	31	6	11	39	297	56	5

Conclusions: Surface chemistry and flow conditions influence the expression level of both ica genes. Hydrophobic biomaterial surfaces and faster flow rate seem to play a crucial role to initial adherence increasing ica gene expression.

P698 In vitro activity of caspofungin and anidulafungin against *Candida albicans* and *C. glabrata* biofilms

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Objectives: Candidiasis is frequently associated with biofilm formation on indwelling medical devices such as central venous catheters, urinary catheters, heart valves and joint prostheses. *Candida* spp. biofilms are less susceptible to antifungal agents than planktonic (free-living) cells. The aim of this study was to test the ability of *C. albicans* and *C. glabrata* to form biofilms and to determine the activity of caspofungin and anidulafungin against free-living cells and biofilms of both species.

Methods: 16 *C. albicans* and 23 *C. glabrata* strains from blood cultures were studied. MICs of planktonic cells were determined following the CLSI M27-A2 document. MIC2 corresponded to the lowest drug concentration that showed a reduction of growth $\geqslant$ 50% compared with the growth control. Biofilms were formed following the method described by Ramage. et al. (1). Antifungal agents at concentrations ranging from 16–0.03 mg/L were added to polystyrene microplates containing *Candida* biofilms of 24 and 48h of maturation. Antifungal activity against biofilms was assessed by the XTT reduction assay. Sessile MICs (SMICs) 50 and 80 corresponded to the lowest drug dilution that supported an OD $\leqslant$ 50% and $\leqslant$ 20%, respectively compared with control.

Results: 10 *C. albicans* and 3 *C. glabrata* developed biofilm (62.5 and 13.04%, respectively). Geometric mean SMIC50s and SMIC80s of caspofungin against *C. albicans* biofilms of 24/48h of maturation were 0.683/1.878 and $\geqslant$16/$\geqslant$16 mg/L, respectively; and against *C. glabrata* 0.038/0.12 and 0.095/0.12 mg/L, respectively. For anidulafungin, these values were: 0.521/4.52 and $\geqslant$16/$\geqslant$16 mg/L against *C. albicans* and 0.095/0.63 and 0.12/1 mg/L against *C. glabrata*.

Conclusions: *C. albicans* produced more biofilm than *C. glabrata*. Caspofungin and anidulafungin showed activity against *C. albicans* biofilms of 24h of maturation, but not against those of 48h. Both echinocandins displayed excellent activity against *C. glabrata* biofilms of 24 and 48h of maturation.

Reference(s)

[1] Ramage G, et al. Standardized method for in vitro antifungal susceptibility testing of *Candida albicans* Biofims. Antimicrob Agents Chemother 2001; 45:2475–2479.

P699 Implication of *Candida* biofilm maturation on the susceptibility to amphotericin B, caspofungin and posaconazole, alone or in combination

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Objectives: *Candida* biofilm-associated infections are a particular challenge in modern implant surgery because of their resistance to conventional therapy. In this work was determined the activity of amphotericin B, caspofungin and posaconazole alone or in combination on biofilms of various *Candida* species at distinct levels of maturation.
Material and Methods: Biofilms of 44 isolates from blood, central venous catheter, urine, skin swabs and medical devices swabs of cardiac surgery patients (*C. albicans* n= 25; *C. glabrata* n = 8, *C. parapsilosis* n = 5, *C. tropicalis* n = 2, *C. krusei*=1, *C. famata* n = 1, *C. norwegiensis* n = 1, *C. guilliermondi* n = 1) were grown for either 24, 48 or 72 hours in 96-well microtiter plates. The biofilms were treated for 48 hours with amphotericin B (AMB), caspofungin (CAS) and posaconazole (POS) alone in increasing concentrations according to the respective MIC (1–128×MIC) and with combination therapy: AMB 0.5 mg/l/CAS 0.5 mg/l or 1 mg/l, and AMB 0.5 mg/l/POS 0.5 mg/l or 1 mg/l. The optical density (OD) was determined using XTT-reduction assay at a wavelength of 450 nm. The OD of the untreated biofilm of the respective isolate was set as 1. To test for viable *Candida* spp. in the biofilms, the unfixed biofilms were scraped off and seeded to Sabouraud agar.
Results: Treatment with CAS reduced significantly the OD of 24- and 72 hours *C. albicans* biofilms at concentrations of 1×MIC ($p < 0.05$), while no significant reduction of the OD of 48 hours old non-*albicans Candida* biofilm was detected up to a concentration of 128×MIC. AMB reduced the OD of 24- and 72 hours biofilm significantly at concentration of 1×MIC – 4×MIC ($p < 0.05$). Significant OD reduction of 48 hours old biofilm achieved AMB at concentrations of 8×MIC (*C. albicans*) and 32×MIC (non-*albicans*). POS was equally effective at all stages of *Candida* biofilms and reduced the OD at a concentration of 1×MIC-2×MIC ($p < 0.05$). Combinations of antifungals led to significant reduction of the OD of the biofilm in the each stage of development ($p < 0.01$).
Conclusion: *Candida* biofilms show different degrees of susceptibility to therapy with a single antifungal agent depending of the phase of development. Particularly 48 hours old biofilms exhibit less susceptibility. Combination therapy attained high OD reduction and may be an option for the treatment of biofilm-associated infections.

P700 Comparison of biofilms formed by clinical oral isolates of non-*Candida albicans Candida* species

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Objectives: Oral candidiasis is a significant problem in patients undergoing treatment for cancer or organ transplantation. Furthermore it is one of the most common and persistent conditions encountered in individuals with human immunodeficiency virus infection and acquired immunodeficiency syndrome. Many Non-*Candida albicans Candida* (NCAC) species, such as *Candida glabrata*, *Candida parapsilosis* and *Candida tropicalis* have recently emerged as significant pathogens of clinical importance. Biofilms have clinical significance as they exhibit high resistance to host defences and antimicrobial agents, therefore representing a persistent source of infectious organisms. Thus, the aim of this study was to compare biofilms formed by different oral isolates of NCAC species.
Methods: A total of 6 strains were isolated from oral tract, including *C. parapsilosis* (n = 2), *C. glabrata* (n = 2) and *C. tropicalis* (n = 2). References strains of each species (*C. glabrata* ATCC 2001, *C. tropicalis* ATCC 750 and *C. parapsilosis* ATCC 22019) were also assayed. Biofilms were analysed after 48 hours through total biomass quantification by crystal violet staining. The ultrastructure of the NCAC species biofilms was observed by Scanning Electron Microscopy. Matrix material was extracted from biofilms and their protein and total carbohydrate contents were determined by the Lowry and Dubois methods, respectively.
Results: The results showed that all NCAC species were able to form biofilms, although there were differences among species and strains. However, *C. glabrata* strains are less capable to form biofilm compared with the other species. *Candida parapsilosis* and *C. tropicalis* strains revealed a multilayer biofilm structure that consists of a dense network of yeast, hyphae and pseudohyphae. On the other hand, *C. glabrata* strains revealed a dense and compact multilayer biofilm structure intimately packed with yeasts without of either pseudohyphae or hyphae. Matrix isolated from *C. parapsilosis* biofilms consisted of higher amounts of carbohydrates but small amounts of proteins. In contrast, matrix from *C. tropicalis* strains consists mainly of proteins, with smaller amounts of carbohydrates. Matrix from *C. glabrata* strains consisted of lower amounts of proteins and carbohydrates.
Conclusions: As a general conclusion, it was possible to infer that clinical oral isolates of NCAC species present different behaviours in terms of biofilm formation, structure and chemical composition.

P701 In vitro anti-biofilm activity of micafungin in a lock strategy

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Objectives: Catheter-related candidiasis are associated with the formation of a fungal biofilm which alters the susceptibility to antifungal agents. Catheter lock therapy could represent a conservative strategy of the catheter in candidaemia involving the static instillation, usually during 12h, of a concentrated solution of antimicrobial agent (100 to 1000 MIC) into the catheter lumen. This study deals with the in vitro anti-biofilm potential of micafungin, used as a lock solution, to manage *C. albicans* biofilms.
Methods: Biofilms of two *C. albicans* strains (ATCC 66396 and ATCC 3153), 12 hours or 5 days old, were formed on 0.5 cm sections of 100% silicone catheters. Micafungin 5 μg/mL (corresponding approximately to 170 MIC) was then added to the biofilms and catheters were incubated for 12h at 37ºC. Micafungin was then removed by washing. The metabolic activity of yeasts within the biofilm was assessed with XTT method, 24h, 48h or 72h after the end of the locks. Controls without antifungal were realised and used as references to calculate the inhibition percentages induced by micafungin locks.
Results: For a young *C. albicans* biofilm (12h), the growth inhibition induced by a 12h micafungin 5 μg/mL lock therapy and observed after a 24h culture without antifungal was at least 67% whatever the studied strain. 48h after the end of the lock, micafungin demonstrated a significant inhibitory activity on the development of 12h old biofilms, reaching at least 54%. 72h after the end of the lock, the inhibition observed was at least 40%.
The efficiency of micafungin lock on the development of a mature *C. albicans* biofilm (5 days old) was also significant: the inhibition observed 24h or 48h after the end of the lock was at least 54% suggesting a persistent efficiency.
Conclusion: These results showed that the micafungin lock treatment was efficient in vitro, on the studied strains, whatever the biofilm maturation stage. This study suggests that this echinocandin could have a real anti-biofilm potential and could become an interesting actor in the lock approach.

P702 CgCDR1, CgCDR2, and CgERG11 gene expression in *Candida glabrata* biofilms formed by bloodstream isolates

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Objectives: A number of molecular mechanisms have been implicated in non-biofilm antifungal resistance in *C. glabrata*, but the relevance of these mechanisms to *C. glabrata* biofilm cells has rarely been examined. We determined whether the expression of azole-resistance genes is altered during the developmental phases of biofilm formation by *C. glabrata*

Methods: Four bloodstream isolates of *C. glabrata* from cases of central venous catheter-related fungaemia showing high in vitro biofilm formation were studied. A microtiter-plate-based colorimetric assay was used to test the fluconazole susceptibility of *Candida* biofilms at 80% inhibition using the 2,3-bis[2-methyloxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide (XTT) reduction assay. The expression of CgCDR1, CgCDR2, and CgERG11 genes during the early (6 h), intermediate (15 h), and mature (48 h) phases of biofilm development, and corresponding planktonic cells, were examined using real-time PCR methods.
Results: The fluconazole minimum inhibitory concentrations (MIC) of *C. glabrata* biofilms formed at 6, 15, and 48 h were all >1,024 ug/ml. The biofilms at 15 h exhibited an approximately 3.3-fold upregulation of CgCDR1 compared with the corresponding planktonic cells, which was higher than the values for biofilms at 6 (1.5 fold) and 48 (0.8 fold) hours ($P < 0.05$). The CgCDR2 transcript levels for biofilms were also highest at 15 h (3.1-fold upregulation), but were minimal at 6 and 48 h (0.5 and 0.8 fold) ($P < 0.05$). By contrast, the CgERG11 transcript levels did not differ among the 6-, 15-, and 48-h biofilms.
Conclusion: Our study demonstrated a temporal increase in the expression of the CDR1 and CDR2 genes in *C. glabrata* biofilms formed by bloodstream isolates during the early biofilm development stage.

P703 New focus – old disease: the paranasal sinuses as possible focus for bacterial lung infections in cystic fibrosis patients

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CF patients often have nasal polyposis and sinusitis because of impaired mucociliary clearance and mechanical obstruction of the sinus ostia by thickened mucus. CF patients who have had a lung transplant are colonised in their new lungs with the same clones as those that were cultured from their old lungs. It has therefore been suggested that the sinuses may serve as a bacterial reservoir after transplantation.
We have genotyped first and subsequent *P. aeruginosa* lung isolates from CF children by using the ArrayTube chip and PFGE. Among first isolates we found a large genetic diversity. We also found that after successful antibiotic eradication of *P. aeruginosa* new colonising isolates had the same genotype as the initial colonising strain. The large genetic diversity in CF children is distinct from most chronically infected patients in our CF clinic where the diversity of strains is more restricted.
Nineteen CF patients (7 males, 12 females, median age: 19 years (range 6–46)) have been treated with Functional Endoscopic Sinus Surgery using CT image guidance to evaluate whether their sinuses serve as a bacterial reservoir for their lung colonisation or infections. Seven patients were chronically infected with *P. aeruginosa*, 2 with *Burkholderia*, 1 with *Achromobacter* and 5 were intermittently colonised with *P. aeruginosa*. We found full or partial agreement between lung and sinus bacteriology in 18/19 (95%) of the patients. Seven patients chronically infected with *P. aeruginosa* have an identical genotype in their lungs for more than 15 years and this genotype was identical to the *P. aeruginosa* obtained from the paranasal sinuses. Four of five intermittently colonised children had growth of the same genotype of *P. aeruginosa* in their sinuses and sputum samples collected up to 2 years prior to the surgery.
In Gram stained smears from the sinuses we found that the bacteria were organised in biofilm structures similar to those seen in the lungs. In sinus smears we only detected few inflammatory cells compared to the lung smears. We think that the colonisation is immunological silent in intermittent colonised patients and cause only an insignificant anti-*P. aeruginosa* antibody response (Th1 response). When the bacteria grow in the lungs a shift towards a Th2 response is seen with high antibody production. In future, we will sample simultaneous sinus and lung cultures in intermittently colonised patients to determine the primary focus for the airway infection.

P704 Adhesion to and biofilm formation on IB3–1 bronchial cells by *Stenotrophomonas maltophilia*: implications in cystic fibrosis

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Objectives: The increasing isolation of *S. maltophilia* from the sputa of cystic fibrosis (CF) patients has become a cause for concern in the CF community, as the organism is resistant to many of the antibiotics prescribed in the management of CF. However, the role of *S. maltophilia* in the physiopathology of CF pulmonary disease has not yet been clarified. The aim of the present study is to characterise the interaction between *S. maltophilia* and CF bronchial epithelium.
Methods: Twelve *S. maltophilia* strains from the sputum of CF patients were tested for adhesion to bronchial epithelial cells isolated from a CF patient (IB3–1; ATCC CRL-2777). IB3–1 monolayers were exposed for 2 h (37ºC, 5% CO2) to *S. maltophilia* 10^8 CFU/ml (MOI: 1.000), then adherence was measured by direct agar plating. The most adhesive strains (TC9 and TC10) were chosen for further characterisation. To determine the contribution of flagella to the ability to adhere to IB3–1 cells, fliI (codifying for the flagellum-associated ATPase) mutants of TC9 and TC10 strains were generated. Furthermore, swimming and twitching motility tests were performed. The significance of the difference ($P < 0.05$) was calculated by ANOVA-test and Neumann-Keuls test.
Results: All isolates tested adhered to IB3–1 cells, although striking differences were observed (mean±SD: $2.1\times10^6\pm1.7\times10^6$ cfu/chamber). In particular, TC9 and TC10 strains (causing monomicrobial and persistent infections) showed an adhesion (5.6×10^6 vs 5.0×10^6 respectively; $P > 0.05$) significantly higher than other strains tested. TC9 and TC10 strains significantly ($P < 0.01$) increased adhesion from 2 to 6 h incubation (TC9: 5.6×10^6 vs 9.3×10^6; TC10: 5.0×10^6 vs 1.1×10^7; 2h vs 6h, respectively). Microscopy showed that after 6h-incubation *S. maltophilia* forms structures consistent with biofilms. Adhesion levels did not correlate with flagellar motility. On the contrary, adherence of flagellar mutants fliI- TC9 and TC10 was significantly ($P < 0.001$) lower than wild-type strains (TC9: 2.5×10^6 vs 5.6×10^6, fliI- vs wt; TC10: 1.7×10^6 vs 5.0×10^6, fliI- vs wt).
Conclusion: Our results suggest, for the first time, that the ability to adhere to CF-derived bronchial epithelium is common in *S. maltophilia* isolates. Flagella function as adhesins in direct binding to IB3–1 cells. The ability showed by *S. maltophilia* to rapidly organise itself as a biofilm could have clinical relevance contributing to antibiotic resistance and bacterial persistence in CF patients.

P705 Mechanisms of environmental persistence of multidrug-resistant *Acinetobacter baumannii* isolates responsible for outbreaks in three Italian hospitals

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Objectives: *Acinetobacter baumannii* (Ab), an environmental Gram-negative bacterium, is considered one of the most dangerous emerging pathogens. The increasing number of outbreaks by clonally related multidrug resistant (MDR) Ab underlines the importance of clonal spread in nosocomial infections. We investigated the mechanisms of persistence in the hospital environment of Ab isolates responsible for outbreaks in three Italian hospitals.
Methods: 52 clinical isolates, collected from 2 acute care hospital and 1 long-term and rehabilitation facility between 2002–2006, were identified and tested for susceptibility with standard procedures. Genotyping was performed by PFGE analysis. Biofilm formation was determined by crystal violet assay. To assess contribution of cellulose production to biofilm formation, cultures were grown in the presence of different concentrations of cellulase.
Results: Genotyping by PFGE showed that most of MDR Ab isolates, susceptible to carbapenems, able to cause recurrent outbreaks in the

studied settings belonged to the same DNA group (clone A), while the others, resistant to carbapenems too, belonged to clone B, which shows close relationship to European clone II. Both clone A and clone B isolates are proficient in biofilm formation, which was favoured by growth in nutrient-deficient medium in comparison to rich medium, and by lower growth temperature. Biofilm formation was inhibited by treatment with the lytic enzyme cellulase in both clones, suggesting that cellulose may act as an important adhesion factor in Ab. However, biofilms formed by clone A were less susceptible to cellulase treatment; in addition, clone A, but not clone B, displays a dark red phenotype on Congo Red-supplemented medium. These observations strongly suggest production of additional cell surface-associated structures involved in biofilm formation in clone A. Finally, clone A showed much stronger resistance to desiccation than clone B.
Conclusions: Our results show that biofilm formation in Ab is favoured in conditions more typical of the external environment rather than of the human host, confirming that biofilm might be involved in the Ab persistence in the environment. We show that cellulose is an important adhesion factor for Ab; however, the clone A identified in our study seems to possess additional biofilm determinants involved in resistance to environmental stresses such as desiccation, and thus potentially important in environmental persistence.

P706 *Aeromonas hydrophila* polar flagellum produces two redundant sodium-driven stator motor proteins

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Aeromonas hydrophila is a ubiquitous water-borne bacterium that has been associated with a wide variety of human diseases, which include gastrointestinal and extraintestinal infections. Mesophilic aeromonads constitutively expressed a single polar unsheathed flagellum and 50%-60% strains can also produce multiple lateral flagella in hifhly viscous environments. Both flagella types are important virulence and colonisation factors.
Aeromonas polar flagella genes are distributed in five non-contiguous chromosomal regions and two of them contain genes whose encoded proteins are homologous to polar flagella stator-motor proteins. Polar flagella region 3 contains pomAB genes, and region 4 contains motX. Only motX mutation abolishes polar flagella motility. These results suggested that lateral flagella stator-motor LafT-U can supply PomA-B function or the presence of a second polar stator-motor involved in polar flagella motility.
The objective of this work is to completely characterise the polar flagella stator motor of *Aeromonas hydrophila* strain AH-3 and correlate polar flagella motility with biofilms formation.
Defined *A. hydrophila* AH-3 mutant in lafTU and a double mutant in both lafTU and pomAB, showed that both mutants are able to swim in liquid media. Then, transposon-insertion mutagenesis using the *A. hydrophila* pomAB mutant as recipient strain, allow us to identified a new chromosome polar flagella region (region 6) containing two polar stator-motor genes named pomC and pomD. *A. hydrophila* PomC and PomD are highly homologous to sodium-conducting polar flagella stator-motors. Distribution of pomAB and pomCD is identical in all the mesophilic *Aeromonas* strains tested, either if the strains were able to produce lateral flagella or not. Furthermore, analysis of *A. hydrophila* pomCD mutants and double mutants in both pomAB and pomCD showed that only double mutants abolish polar flagella motility.
Swimming motility assays in presence of different amiloride and sodium chloride amounts with *A. hydrophila* AH-3 and polar flagella stator-motor mutants demonstrate that PomA-B and PomC-D polar flagella stator-motor are sodium dependents. Biofilm assays showed that polar flagella motility is involved in biofilm formation.
In conclusion we demonstrated that PomA-B and PomC-D are redundant set of sodium-driven stator motor proteins, as none of them by separate is essential for polar flagella motility neither in aqueous nor high-viscosity environments.

P707 The AcrAB-TolC efflux system contributes to biofilm formation in *Salmonella enterica* serovar Typhimurium

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Objectives: To determine the role of the AcrAB-TolC efflux system of S. Typhimurium in biofilm formation.
Methods: Strains used were S. Typhimurium 14028 and isogenic derivatives lacking acrAB or tolC. Biofilms were grown in defined minimal media in microtitre trays or transwell trays and quantified by staining with 1% crystal violet and measurement of absorbance using a FLUOstar optima plate reader. RNA was prepared using the SV total RNA kit (Promega, U.K). Gene expression was determined using comparative RT-PCR and denaturing HPLC.
Results: Mutants lacking either acrAB or tolC were compromised in their ability to form biofilms compared to 14028. L829 (14028, tolC::aph) produced 3 fold less biofilm compared to 14028, L830 (14028, acrAB::aph) was further compromised in its ability to form biofilm and produced 10 fold less biofilm than 14028. Co-incubation of mutants lacking acrAB or tolC and 14028 separated by a membrane which acts as a barrier to cells but not solutes in transwell assays showed no rescue of biofilm formation. This suggested that the biofilm defect of L829 and L830 can not be alleviated by a molecule(s) secreted by wild-type strains. RT-PCR of acrB and tolC demonstrated that both genes were over-expressed (2-fold) by cells in a biofilm relative to cells grown in planktonic culture.
Conclusions: A functional AcrAB-TolC efflux system is required for normal biofilm formation in S. Typhimurium and expression of this system is increased during growth as a biofilm. It was not possible to rescue biofilm formation in the transwell experiments suggesting that AcrAB-TolC does not export a structural molecule required for creation of a biofilm. The data presented here demonstrate that AcrAB-TolC is required for biofilm formation as well as intrinsic resistance to many antimicrobials and pathogenicity as has previously been described.

P708 Differences in biofilm development and antibiotic susceptibility among human clinical Ureaplasma sp.

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Objectives: The aim of this work was to investigate the ability of clinical isolates of Ureaplasma sp. to be biofilm formers, comparing the antibiotic susceptibility between sessile cells (minimum biofilm inhibitory concentration, MBIC) and their planktonic counterpart (conventional MIC).
Methods: A total of 11 Ureaplasma sp. isolates were obtained from clinical samples that included urine, semen, and urethral exudates from urethritis or chronic prostatitis, and also from urine of two healthy volunteers. All isolates were recovered during 2007 at the Service of Microbiology of the University Hospital Ramón y Cajal, in Madrid, Spain and corresponded to unrelated patients. *Mycoplasma* identification was carried out by growth in 10C broth medium and growth of typical brown colonies on A7 medium by urease production in the presence of manganese chloride. Antibiotic susceptibility was determined by broth microdilution method. Biofilm susceptibility assay was performed in 96-well microtiter plates, using the 10-C UREA-medium pH 6. Bacterial biofilms was investigated by immersing the pegs of a modified polystyrene microtiter lid into this biofilm growth plate until the media turned from orange to fuchsia. Comparison between MBIC50-MIC50 and MBIC90-MIC90 values was carried out only in strains able to form biofilm.
Results: Positive biofilm formation was observed in 9 isolates (81.2%); being the two biofilm non-formers isolates obtained from urethral exudates. Taking into account the antibiotic susceptible-resistant concept, most Ureaplasma isolates were susceptible to all antibiotics used when the traditional MIC test were applied. However, the resistant isolates increased considerably when the antibiotic susceptibility test was carried out in biofilm formation. In general, MBIC were one or two dilution

higher than MIC, although there were some exceptions in which the MBIC was lower. These contradictory results were observed in four strains for telythromycin, levofloxacin, and tetracycline, observing a higher susceptibility for these antibiotics in the biofilm formation than in the planktonic one. Nevertheless, comparing the MIC50–90 with the MBIC50–90 in all biofilm-formers strains, MBIC values were higher for all antibiotics.
Conclusion: Ureaplasma sp. isolates from clinical human samples are able to growth in biofilm formation showing reduced antibiotic susceptibility compared with the planktonic one; although for some antibiotics MBICs are lower than MICs.

P709 Association between biofilm formation and antibiotic resistance by *Salmonella enteritidis*

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Non-typhoidal salmonellosis in humans is usually a self-limiting disease confined to the gastrointestinal tract, but when infection spreads beyond the intestine, or when immunocompromised persons are affected, effective antimicrobial treatment is essential. Besides, numerous studies have shown that *Salmonella* spp. are capable of adhering and forming biofilm on diifferent surfaces and under different environmental conditions. The aim of this study was to analysed the association between antibiotic resistance and biofilm formation between tested strains of Salmonella Enteritidis.
A total of 30 strains of Salmonella Enteritidis were isolated from patients with acute diarrhoea at the Institute of Infectious and Tropical Diseases in Belgrade during 2002. The strains were identified and serotyped by conventional methods. Antimicrobial susceptibility testing for 11 antibiotics was performed according to the CLSI recommendations. The quantification of biofilm formation was performed by modified microtiter-plate test. Differences in the quantities of produced biofilm were examined by Friedman test, followed by the Wilcoxon signed ranks test. Statistical analysis of relationship between antibiotic resistance and biofilm formation was examined by Spearman coefficient of correlation.
Fully susceptibility to all antibiotics was established in 10 (33.3%) isolates, and 20 (66.7%) strains were intermediary susceptible or resistance to one or more antibiotics All isolates were sensitive to cefotaxime, gentamicin and ciprofloxacin. 13 (43.3%) isolates were resistant or intermediary susceptible to tetracycline, 8 (26.7%) isolates were resistant to nalidixic acid, ampicillin, and amoxicillin-clavulanic acid, while 3 (10%) were resistant to trimethoprim-sulfamethoxazole, chloramphenicol and kanamycin, and 2 (6.7%) was intermediary susceptible to cefuroxime. Among 27 (90%) biofilm producing isolates, 21 (77.8%) were weak, 2 (7.4%) was moderate, and 4 (14.8%) were strong biofilm producers. Antibiotic resistance was more frequently seen in biofilm-positive than in biofilm-negative isolates ($p < 0.05$). Statistical analysis showed correlation between resistance to antibiotics and the quantity of biofilm by bifilm producing strains of Salmonella Epidermidis.
Biofilm-positive isolates of Salmonella Enteritidis were more resistant to antibiotics than biofilm-negative isolates. Obtain results found an association between biofilm production and antibiotic resistance.

P710 Impact of antibiotics on biofilm and some other properties of *Klebsiella pneumoniae* in vitro

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Objectives: *K. pneumoniae* is a pathogen responsible for different human infections and it is also involved in outbreaks of nosocomial infections. Antibiotic resistance of these bacteria have become a serious clinical problem. The effects of subinhibitory concentrations (sub-MICs) of ciprofloxacin, enoxacin, norfloxacin, gentamicin and netilmicin on biofilm formation, hydrophobicity, lipase and response to oxidative stress in two clinical *K. pneumoniae* strains (39, 61/P) were evaluated.
Methods: Microtiter plate assay for biofilm formation, adherence to xylene for hydrophobicity, spectrophotometric method with Tween 20 for lipase activity and effect of H2O2 for response to oxidative stress were applied.
Results: Biofilm production by the strain 39 treated with quinolones and aminoglycosides and by the strain 61/P with aminoglycosides was markedly decreased. The most effective concentrations (1/2 of the MICs) suppressed biofilm formation to 28.7–56.9% of the control levels. Quinolones to the lesser extent altered biofilm in strain 61/P. Though antibiotics in the most cases enhanced bacterial adherence to xylene, the strains remained in the hydrophilic state. Decreased lipase activity to the different range in the strains treated with all concentrations of quinolones (with the exception of norfloxacin and enoxacin in strain 39) and netilmicin and with some concentrations of gentamicin was found. Antibiotic treated bacteria in most of the cases showed higher sensitivity to oxidative stress (with the exception of norfloxacin), but in case of netilmicin in strain 39 no changes were observed.
Conclusion: *K. pneumoniae* treated with antibiotics manifested suppressed biofilm production and also altered adherence to xylene, lipase and response to oxidative stress in dependence on strain, type and concentration of antibiotic.
This work was supported by Ministry of Health of the Slovak Republic under the project Analysis of biofilm production in nosocomial strains as a basis to prevent infections in healthcare institutions 2005/24-SMU-02.

Bacterial CNS infections

P711 Rapid decrease of intrathecal cortisol levels correlates with severity of bacterial meningitis

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Objectives: Bacterial meningitis (BM) still represents an infectious disease with a significant morbidity and mortality. We have previously demonstrated that the elevated cortisol concentrations in the cerebrospinal fluid (CSF) are associated with the severity of BM and can also serve as useful diagnostic marker for differentiation from viral meningitis [Crit Care 2007]. Therefore, the present study was aimed at description of the kinetics of CSF cortisol levels during BM and assessment of its relationship to severity and distinct bacterial aetiology of the disease.
Methods: A total of 55 patients with BM were enrolled in the study. The BM severity was evaluated using APACHE II (Acute Physiology and Chronic Health Evaluation), SOFA (Sequential Organ Failure Assessment) and GCS (Glasgow Coma Score) scoring systems. Paired samples of CSF and serum were obtained on admission and 3–5 days later. Concentrations of cortisol were determined by radioimmunometric assay. Statistical analysis was performed using SPSS software^{TM}.
Results: Bacterial aetiology was confirmed in 42 BM patients (76%): *Neisseria meningitidis* in 17 cases (40%), *Streptococcus pneumoniae* in 14 cases (33%) and 11 cases were due to other bacteria. Increased CSF cortisol correlated with serum cortisol on admission as well as follow-up ($r = 0.60$, $p < 0.001$; $r = 0.78$, and $p < 0.001$, respectively). In addition, CSF cortisol levels on admission were associated with APACHE II ($r = 0.77$, $p < 0.001$), SOFA ($r = 0.68$, $p < 0.001$) and GCS ($r = -0.60$, $p < 0.001$). Also, CSF cortisol demonstrated a decrease between the initial and follow-up lumbar puncture ($p < 0.001$). Moreover, this decrease correlated with APACHE II ($r = 0.53$, $p < 0.001$), SOFA ($r = 0.51$, $p < 0.001$), and GCS ($r = -0.46$, $p < 0.001$). Although CSF cortisol concentrations were higher in pneumococcal meningitis compared to meningococcal meningitis, this difference was insignificant ($p = 0.12$).
Conclusion: Our results demonstrate that the initially elevated CSF cortisol levels rapidly decrease after administration of BM therapy (i.e. antibiotics and dexamethasone). Moreover, this reduction was more pronounced in patients with severe course of BM. The tendency of the association between the high CSF cortisol levels and pneumococcal aetiology of BM observed in our study should be further evaluated on a larger cohort of patients.
Acknowledgment: The study was supported by the grant IGA NR/9316–3.

P712 Spontaneous Gram-negative bacillary meningitis in adult patients

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Objectives: The aim of this study was to describe the incidence, predisposing factors, aetiology, clinical features, complications, treatment and outcome of adult patients with spontaneous Gram-negative bacilli (GNB) meningitis (GNBM) during the last 25 years in a tertiary hospital.
Methods: We prospectively collected all cases of spontaneous bacterial meningitis occurring in adult patients at the Hospital de la Santa Creu i Sant Pau (Barcelona) between 1982 and 2006. Cases due to *Haemophilus influenzae* were excluded.
Results: Six hundred sixty-four patients with bacterial meningitis were registered. Forty of them were caused by GNB (6%). Twenty-three occurred in the first 12 years (56%) and 17 in the last 12 years (42%) (p= 0.27) with an overall incidence of 2 cases/100.000 patient-year. There were 23 (58%) men and the mean age was 63 years (SD 16 years). Underlying conditions were present in 29 patients (73%). An extrameningeal focus was found in 30 cases (75%): urinary infection in 13 patients (43%), and otitis/sinusitis and abdominal focus in 4 patients (13%), respectively. Nine episodes (22%) were nosocomial acquired.
The classic triad of meningitis (altered mental status, fever and neck stiffness) was found in 13 patients (32%). *Escherichia coli* was the most common cause (15 patients [38%]), followed by *Pseudomonas* spp. in 10 patients (24%). However, *Pseudomonas* spp. was the leading cause in nosocomial cases.
During the clinical course, neurologic complications occurred in 20 patients (50%) and systemic complications in 27 patients (68%). The overall mortality rate was 53% (21 patients). We found no significant difference ($p=0.678$) in mortality rates between two periods. To have underlying diseases, urinary infectious (versus other infectious focus) or developing acute renal failure were independent factors associated with a higher mortality (OR 9.8 [CI95% 1.2–77.6]; OR 8.7 [CI95% 1.2–64.3]; OR 11.7 [1.4–99.8], respectively).
Conclusions: Currently Gram-negative bacilli are an unusual cause of GNBM, accounting for 6% of all spontaneous meningitis in adults. No differences in the incidence were found over the 25-year period of study. Most of the patients have underlying diseases and this is a risk factor of higher mortality. The mortality rate is high (more than fifty percent of cases) and seems not to have decreased in the last years.

P713 Clinical features and prognosis factors of community-acquired acute bacterial meningitis in Spain

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Objective: We conducted a multicentre study to determine clinical features and prognostic factors of community-acquired acute bacterial meningitis (ca-ABM) in nine hospitals of the Spanish Network for Investigation in Infectious Pathology (REIPI)
Methods: From Nov 2003 to Sept 2006 all patients >29 days diagnosed of ca-ABM were prospectively evaluated. The diagnosis was based on clinical condition, chemical and microbiological results in cerebrospinal fluid (CSF) and/or blood. Outcomes were classified as favourable or unfavourable (death or cure with disabilities). Predictors were identified through logistic-regression analysis.
Results: Four hundred and fifty five patients were included, 56% were men, the median age was 42 years (range: <1–91), *Streptococcus pneumoniae* (Sp) 43% were the most common aetiology agent followed to *Neisseria meningitidis* (Nm) 30%, *Listeria monocytogenes* (Lm) 13% and others 14%. Nm was the most common pathogen among patients $\leqslant$ 18 years (45.9%) and Sp in >18 years (80.7%). Predisposing conditions were oto-mastoiditis or sinusitis in 20% of the cases, previous immunosuppression (16.7%), diabetes (12.4%), CSF leak (8.5%) and alcoholism or cirrhosis (8.5%). The triad of fever, neck stiffness and altered mental status was present in 43.7% of the episodes. Rash was present in 20% of the cases, 12% had focal neurological deficits and 9% seizures. Penicillin susceptibility was tested in 146 Sp strains; of which 21.2% showed intermediate resistance (IR) and 4.8% resistance (R); of 119 Nm strains 6.7% presented IR to cefotaxim. Ampicillin susceptibility was tested in 39 Lm strains and all were sensible. Outcome was unfavourable in 26% of the cases with an overall mortality of 14%. In the multivariate model the unfavourable outcome risk factors appeared: age $\geqslant$65 years, respiratory failure, neurological deficits and seizures during treatment.
Conclusions: The most common pathogens causing ca-ABM in our area are Sp, Nm and Lm. Unfavourable outcome is high, so particular attention should be done to people with identified risk factors.

P714 Community-acquired acute bacterial meningitis in older people in Spain

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Objective: Older age is considered to be an important risk factor for unfavourable outcome in community-acquired Acute Bacterial Meningitis (ca-ABM). The aim of this study is to describe clinical characteristics and outcome of ca-ABM in patients over 65 years compared to younger ones
Methods: A prospective multicentre study of ca-ABM was carried out in 9 Spanish hospitals included in the REIPI programme (Spanish Network for the Research in Infectious Diseases) between 1/11/2003 and 30/07/2006. Patients were diagnosed on the basis of a compatible clinical, biological and microbiological data. An adverse clinical outcome was defined as death or cure with disabilities.
Results: 415 patients were diagnosed with ca-ABM, 293 <65 years-old (median age: 29.8, range: 0–64) and 122 $\geqslant$65 years years-old (median age: 74.4, range: 65–91). Among patients over 65 years *Streptococcus pneumoniae* (Sp) was the most frequent aetiology (44.3%), followed by *Listeria monocytogenes* (Lm) (20.5%). Lm was significantly more frequent in this group compared to younger patients (20.5% vs 7.5%, $p<0.05$) among which *Neisseria meningitidis* (Nm) was the most common aetiology (12.3% vs 32.1%, $p<0.05$). Diabetes (28% vs 6%, $p<0.05$) and a distant foci of infection (12.7% vs 3.9%, $p<0.05$) were more frequent in elderly who more often presented the classic triad of fever, neck stiffness and altered mental status (50% vs 39%, $p=0.021$), a GSC (Glasgow Score Come) <14 (79.3% vs 50.2%, $p<0.05$), focal neurological deficits (21.2% vs 11%, $p=0.007$) and respiratory failure (23.7% vs 11%, $p<0.05$). Elderly cohort was treated with third-generation cephalosporin associated to ampicillin in 41% of the cases compared to only 18% of the cases in patients under 65 years ($p<0.05$). Among patients over 65 years hemodynamic failure (25.4% vs 12%, $p<0.05$), a GSC decrease on evolution (26.3% vs 13.8%, $p<0.05$) and an adverse clinical outcome (45% vs 20%, $p<0.05$) were significantly more frequent. Mortality rate was higher among adults over 65 years (29% vs 8%, $p<0.05$).
Conclusions: ca-ABM in patients over 65 years is predominantly due to Sp followed by Lm which is significantly more frequent in this group. Ca-ABM in the elderly cohort significantly present with more severe clinical features and adverse evolution being overall mortality in this group significantly higher.

P715 Secondary cases of meningococcal disease can be prevented by the immediate identification and eradication of the disease-causing strain of *Neisseria meningitidis* in close contacts

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Objective: To prevent secondary cases of meningococcal disease by the identification and eradication of the disease-causing strain in close contacts of the primary case.

Methods: Following the bacteriological verification of invasive meningococcal disease, the local physician is informed and "close contacts" (household members, kissing contacts, classmates, and playmates, children in the same kindergarten and others who have slept in the same room as the patients during the last 2 weeks before admission) are identified. On the same day or the following day, throat specimens are collected, plated and incubated at 37ºC in 10% CO_2. Upon growth the next day of oxidase-positive Gram-negative diplococci, colonies are picked and DNA extracted. A PCR amplicon restriction endonuclease analysis (PCR AREA) with primers from the folP-gene is performed. When the PCR AREA band pattern is identical with that of the disease-causing strain, contacts are given rifampicin.
Results: During the period 1988–2007 there have been 66 primary cases with meningococcal disease in the County of Telemark (165.000 inhabitants). No secondary case has been seen. The mean incidence of invasive meningococcal disease in all Norway during the first 10-years period (1988–1997) was 3.63/100,000 inhabitants/year (CI: 0, 52) and fell to 1.39 (CI: 0, 48) during the last period (1998–2007). The corresponding figures for the County of Telemark were 3.41 (CI: 1, 35) and 0.84 (CI: 0, 50), and in the 3 neighbouring counties the figures were 2.89 (CI: 2, 89) and 1.74 (CI: 0, 62). The disease-causing strain was found in 70 (3.1%) out of a total of 2252 contacts. The prevalence of the disease-causing strain was higher (14.8%) in household members and kissing contacts than in all other groups combined (1.7%). The odds ratio for carrying the disease-causing strain in household-members and kissing contacts was 8.74.
Conclusion: Secondary cases of meningococcal disease may be prevented by the rapid identification of the disease-causing strain in close contacts followed by subsequent eradication with rifampicin. Household-members and kissing contacts have the highest risk of carrying the disease-causing strain.

P716 Short-course therapy for adult meningococcal sepsis and/or meningitis: 23-year experience

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Objective: Short course therapy for meningococcal disease have been previously shown as effective as longer therapies. In order to reinforce our results in adults we present our accumulated experience in 138 patients (pts) treated with a short course therapy for systemic meningococcal disease.
Patients and Methods: All pts admitted to our hospital with systemic meningococcal disease (positive blood or CSF culture or meningitis with petechial purpura) from January 1984 have been scheduled to a short 4 days antibiotic therapy and have been compared with the historical 7 days treated pts. Data were prospectively recorded in a 128 variables protocol.
Results: 138 adult pts (37 M/101 F) mean age 45.5±20 (18–87) were evaluated. Mean duration of therapy was 4.9±2 days, median 4 days. 17 pts presented shock on admission, 38 pts were treated with penicillin, 90 with ceftriaxone, 5 with cefotaxime and 4 with other drugs. Mortality was 13/138 (9%) due to early sepsis in 5 pts, neurological complications in 1 and late causes in 7. Mild neurological sequelae were present in 15 pts (12%). No relapses were observed after one month follow-up. When compared with pts treated previously with a 7 days or longer therapy course, 129 pts, (36 M/93 F) mean age 43.8±19 (18–80), mean duration of therapy 7.22 days, there were no differences in positive blood cultures, shock on admission (25 pts) mortality 12/129 (9%), due to early sepsis in 9 pts and neurological complications in 3, relapse (no cases) or sequelae 12 (10%) (NS). Antibiotic therapy in this long course therapy group consisted in penicilIin 119 pts, ampicillin 4 pts and chloramfenicol 6 pts.
Among short course therapy pts, in the subgroup of 16 pts presenting as sepsis without meningitis (1M/15 F) mean age 47.38±22 (18–82) with shock on admission in 3 pts treated for 4.6±2 days there were a mortality of 1/16 (6%), no relapses and sequelae were present in 1 patient without differences with the previously long-therapy treated pts. In the subgroup of 10 pts presenting as severe sepsis (hypotension or shock + thrombopenia + coagulation disorder with or without meningitis) with mean age 49.6 (21–73), shock in 7/10 and disseminated intravascular coagulation in 8/10 mortality was 3/10 (30%) all of them in the first day of therapy.
Conclusion: Short course (4 days) antibiotic therapy in adult meningococcal disease either meningitis or sepsis (including severe sepsis) is a safe therapy without relapses.

P717 Epidemiology of bacterial meningitis during a 5-year period in an infectious diseases unit in northern Italy

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Objective: To evaluate the frequency of isolation of *S. pneumoniae* (SP), *N. meningitidis* (NM), *H. influenzae* (HI), *L. monocytogenes* (LM) and other bacteria during meningitis, also defining their patterns of antibiotic susceptibilities, in order to optimise both prevention strategies and antibiotic therapy of this severe infection.
Methods: During a 5 years period (January 2002–June 2007) we prospectively evaluated patients affected by meningitis, in particular we collected their demographic data, the presence of co-morbidities and meningeal infection's predisposing factors. Liquor biochemical characteristic together with microbiological results were reported; the susceptibility test were performed by Kirby Bauer method.
Results: During the study period we collected 31 bacterial meningitis (BM), increasing from 2002 (4 cases) to 2006 (10 cases), with this trend confirmed during the first half of 2007 (6 cases). During the first three years period we found only SP as causal agent, but from 2005 NM has appeared and become predominant in relationship with other bacteria. During the study period we had only two LM (in 2002 and 2006) and one HI (2006). SP has never found to be penicillin resistant but in 25% of cases it showed an intermediate susceptibility to this antibiotic. 80% of NM was resistant-intermediate to penicillin. The only isolated HI didn't produce β-lactamases.
Conclusions: In our Institution BM is becoming more frequent than in the past, and this disagree with national data which show a trend in reduction from 2006. In particular we noted an increasing NM's frequency of isolation, with a predominance in last 3 years if compared with SP, which remained quite constant. This data are too limited to be interpreted as an outbreak but they call our attention to preventive interventions such as vaccination for this severe infection.
In contrast with European Antimicrobial Resistance Surveillance System data, the frequency of isolation of penicillin-resistant SP is low, but we frequently found penicillin resistant-intermediate NM. These antibiotic patterns support our empirical antibiotic approach to BM with ceftriaxone (2 gr bid i.v.) in association with levofloxacin (500 mg bid i.v.) which shows in vitro activity against LM as well. The effectiveness of this antibiotic treatment is proved by the mortality for BM as well, that was 0% during the study period.

P718 Laboratory-confirmed meningococcal disease in children over a 10-year period in Athens, Greece

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Objectives: Meningococcal disease (MD) may be presented clinically as septicaemia, meningitis, or both. Our study aimed to review the epidemiological data of MD, and to assess the phenotypes of *Neisseria meningitidis* in the paediatric population admitted to "P. & A. Kyriakou Children's Hospital" over a 10-year period.
Methods: The study population included patients with: (i) suspected invasive infection, meningitis and/or septicaemia and (ii) blood and/or CSF samples positive for *N. meningitidis*. All cases were laboratory-confirmed by culture and/or PCR. Culture, identification and serogrouping was carried out by standard methods. Susceptibilities to antimicrobials were determined by Etest. All isolates were sent

for serotyping and serosubtyping to the Hellenic National Meningitis Reference Laboratory. In addition, CSF and/or blood samples were also subjected to PCR for the detection of meningococcal DNA.
Results: During the study period, 235 cases of MD were recorded in patients (135 boys, 100 girls) aged from 15 days to 14 years (mean 5.1y±3.7SD). The clinical diagnoses were: meningitis associated with septicaemia (41.3%), septicaemia (40.0%), and meningitis (18.7%). In 150 cases (64%) the diagnosis was confirmed by PCR. The annual distribution of cases was: 15, 13, 39, 44, 53, 30, 15, 9, 10, 7 for the years 1998–2007, respectively. Most of cases (66%) occurred during the winter and early spring. Serogroup B accounted for 44.26% (104/235); serogroup A for 12.77% (30/235); serogroup C for 7.23% (17/235); serogroup W135 for 6.81% (16/235); serogroup Y for 0.85% (2/235); while non-serogroupable remained 28.09% (66/235). Serotype and subtype was determined in 72% of the isolates. A high differentation was observed among the phenotypes belonging to serogroup B. Phenotype B.4:P1.14 was most predominant (23.6%). All isolates were found to be susceptible to cefotaxime, and 82% to penicillin. Fully resistant strains were not found.
Conclusions: The epidemiological pattern of MD studied cases is similar to that found elsewhere in Europe. In contrast, the phenotype B.4:P1.14 (ST-269) is rarely found among the meningococcal strains isolated in other European countries. PCR is a valuable tool for the rapid diagnosis of meningococcal disease, as antibiotic prior sampling may reduce the diagnostic efficacy of the culture.

P719 Acute bacterial meningitis in an Athens childrens hospital over an 8-year period (2000–2007)

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Objective: this study describes the epidemiological and clinical features of bacterial meningitis in children for an 8-year period (2000–2007) in a paediatric hospital.
Materials and Methods: A total of 1630 hospitalised children with suspected clinical symptoms and signs of meningitis, aged between 16 days to 14 years, underwent a lumbar puncture. CSF and blood samples were investigated by laboratory methods such as white cell count in the CSF as well as cultures. In addition, all samples (blood and CSF) presenting >50 cells of polymorphonuclear type, were investigated by a multiplex polymerase chain reaction (PCR) for *N. menigitidis* (N.m), *H. influenzae* type b (H.inf) and *S. pneumoniae* (S.pn.) at the National Meningitis Reference Laboratory. All isolated strains were identified by using classical methods. Susceptibility test was performed by E-test according to CLSI guidelines. Serogroups were determined by slide agglutination test.
Results: Seventeen percent (17%, 272/1630) were found positive for meningitis; 219 (80.5%) had lymphonuclear predominance and 53 (19.5%) polymorphonuclear. Proven bacterial meningitis was diagnosed in 53 children with median age of 3–5 years. Males (157/272, 58%) outnumbered females. The most prevalent microorganism causing bacterial meningitis was N.m (n = 43, 81%) followed by *Enterococcus faecalis* (n = 3, 5.6%), S.pn (n = 2, 3.8%), *S. agalactiae* (n = 2, 3.8%), Enterobacteriaceae (n = 2, 3.8%) and *S. pyogenes* (n = 1, 2%). A high percentage (40%) of patients developed bacteraemia. Of the 43 meningococcal cases, 6 (14%) CSF samples were CSF culture and PCR positive while 37/43 (86%) cases were only PCR positive. Of those 23 (62%) were blood positive, 10 (27%) CSF and the remaining 4 (11%) were PCR positive for blood and CSF. The most predominant serogroup was B (53.5%), followed by A (14%), W135 (11.6%), C (2.3%) while 18.6% remained non-groupable (NG). The phenotype 4:P1.14 predominated among the serogroup B strains. The majority of S.pn strains belonged to serotype 18C. All strains were susceptible to antibiotics. Most cases occurred during winter (November-March).
Conclusions: After the use of vaccines (H. inf type b, N.m and S.pn) the frequency of bacterial meningitis has been decreased. N.m serogroup B:4:P1.14 was the prevalent cause of bacterial meningitis. No cases of H.inf meningitis was recorded. The use of molecular techniques contributed significantly towards more accurate and rapid recognition of bacterial meningitis infections.

P720 Epidemiology, clinical presentation and prognostic factors for death of bacterial brain abscess: a twelve-year review

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Objective: Despite the advent of modern neurosurgical techniques, new antibiotics, and powerful imaging technologies, brain abscess remains a potentially fatal central nervous system infection. The aim of this study was to determine the epidemiological trends, prognostic factors, and outcomes of bacterial brain abscess.
Methods: Over a period of 12 years (1992–2005), 71 patients were retrospectively identified as having brain abscesses in a tertiary referral hospital in northern of Spain. Quantitative data are expressed as Mean (SD) and qualitative data are expressed in number and/or percentage (%).
Results: The mean age of the 71 patients was 45.2 years (10.6). There was a male predominance (73% of cases). Fever (28 patients, 30%), focal neurological symptoms (27 patients, 30%) and headache (19 patients, 26.8%) were the main clinical manifestations. The frontal lobe was the commonest anatomical site (28 patients, 41.2%). Bacteria were isolated from 33.8% of patients and monomicrobial infections occurred in 18 (37.5%) patients. No microorganisms were isolated in 23 (47.9%) patients. Staphylococci accounted for 33.5% of isolates and were the prevalent pathogens associated with haematogenous spread. Head and neck infections, neurosurgical procedures and immunosuppression were the main predisposing factors. Twenty seven (40%) patients underwent surgical procedures and 39 (57.4%) patients need anticonvulsive therapy. Fifteen (21%) patients died, most of them (12 patients, 29.3%) received only medical treatment. The most important univariate predictors of mortality were the presence of immunosuppression (P= 0.003), multiple organ failure in the ICU (P = 0.001), medical treatment alone (P = 0.05), multiple abscesses (P = 0.05), older age (p= 0.02), and the APACHE II score in the ICU. Logistic regression analysis showed that multiple organ failure (odds ratio [OR], 30.7; 95% confidence interval [CI] 1.8 to 514.1), multiple abscesses (OR 3.7; 95% CI 1.0 to 14.2), and delay to start antibiotic treatment after the diagnosis (OR 1.5 per day; 95% CI 1.1 to 2.1) were associated with a high mortality.
Conclusion: Despite the availability of new antibiotics and the development of better neurosurgical techniques, mortality of brain abscess is still high. Multiple organ dysfunction, abscess number, and delay to start antibiotic treatment after the diagnosis were associated with higher mortality.

P721 Is vancomicin necessary in empirical treatment of community-acquired meningitis?

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Introduction: Community Adquired meningitis is an important disease in our emergency departments. Empirical treatment is necessary in order to improve prognosis. National guidelines (SEIMC) include vancomicin combined with cefotaxime as first election therapy.
Objective: To study susceptibility to different antibiotics in community adquired meningitis reviewing initial empirical treatment, and determine whether the introduction of vancomycin in the empirical treatment provides some benefit
Methods: a descriptive retrospective study reviewing medical reports and microbiological cultures in patients admitted in the Emergency room of the Hospital Universitario del Mar, Barcelona (455 beds, and an area of influency of 500000 habitants) during 1 year (june 2006 to june 2007).
Results: 19 patients were admitted with community-adquired meningitis. 10 men and 9 women. Mean age 52 (range 23–78).

The most common causative organisms were *S. pneumoniae* 9 (47.3%), N. meningitidis 7 (36.8%) and *L. monocytogenes* 3 (15.9%).
Among *S. pneumoniae*, only 1 strains was resistant to Penicillin (Minimum inhibitory concentration (MIC) >0.1 μg/ml) but continued sensibility to cephalosporin and vancomicin. The rest of isolated strains had MIC < 0.5 μg/ml to cephalosporin group. Of *N. meningitidis* in 1 case was resistant to penicilin (MICs >1 μg/ml) and showed fully sensibility to cephalosporines and vancomicine. No resistance to tested antibiotics was observed in *L. monocytogenes*.
In the Emergency Department cefotaxime and vancomicin as empirical treatment was initiated in 11 patients (57%). Four patients died (all of them with combined treatment), from whom 3 had pneumococcical meningitis and 1 with meningococcical menigitis.
Conclusion: *S. pneumoniae* is the most frequent pathogen in community adquired bacterial meningitis in our population. All isolated *S. pneumoniae*, *N. meningitidis*, *L. monocytogenes* strains remained susceptible to currently used antibiotics in empirical treatment. Therefore, adding vancomicin as empirical treatment in community adquired meningitis does not improve any benefit, and theoretically only adds potential side effects and increased costs. However, monitoring the resistance rates in our community is necessary in order to adapt clinical guidelines.

P722 Scrub typhus central nervous system complications

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Background: *Orientia tsutsugamushi* spreads throughout the body via the bloodstream and lymphatics, and induces vasculitis leading to symptoms of systemic organ invasion.
Among these, meningoencephalitis is a life-threatening manifestation.
Methods: We conducted a prospective study of potential scrub typhus patients who were admitted to Chosun University Hospital between September 2004 and December 2006 to investigate the clinical and laboratory features of patients with scrub typhus meningoencephalitis and the therapeutic outcomes, and to determine the factors associated meningoencephalitis
Results: Multivariate analysis demonstrated that the presence or absence of pneumonia was significantly associated with the occurrence of scrub typhus meningoencephalitis (OR, 8.9; $P < 0.001$; CI, 2.9–27.2). Although appropriate antimicrobials such as doxycycline agents were administered at an early stage, meningoencephalitis still occurred.
Conclusions: Physicians should be aware of the possibility that meningoencephalitis could be developed during the course of appropriate drug therapy such as doxycycline. Close observation and intensive care are essential for patients with factors associated meningoencephalitis, particularly those with pneumonia. Increasing the dosage of doxycycline or administering antimicrobial agents such as rifampin with good penetration to the central nervous system, should be considered in such cases.

P723 Neurological complications of Mediterranean spotted fever

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Background: Mediterranean spotted fever (MSF), a systemic *Rickettsia conorii*, is endemic in the Mediterranean region. It thought to be a benign disaese, but in 5–10% of cases, it is responsable for severe systemic manifestations. Neurological involvement has been reported in 28% of cases.
Objective: The aim of this study is to describe demographic characteristics, clinical presentation and outcome of neurological complications of MSF.
Methods: we conducted a retrospective study of patients who developped MSF with neurological involvement between January 1992 and June 2007 and admitted in the department of infectious diseases of Rabta Hospital in Tunis. The diagnosis of MSF was based on clinical criteria and/ or positive rickettsial serology.
Results: 16 patients with neurological manifestations of MSF were included, 15 male and 1 female. The mean age is 42.7 years. 9 patients are living in rural area and 10 owned dogs. Typical clinical signs are: fever (100%), headache (43.5%) and maculopapular rash (75%), inoculation eschar (62.5%). Neurological involvement includes: 7 meningitis (44%), 5 encephalitis (31%), 3 meningoencephalitis (19%) and 1 case of facial nerve palsy (6%).
Biochemical laboratory tests revealed: normal white blood cell count in 8 cases (50%), thrombocytopenia in 7 cases (44%), hepatic cytolysis in 10 cases (62.5%). A high level of lactate dehydrogenase is noted in 10 patients (62.5%). Lumbar puncture was performed in 14 cases. Analysis of cerebrospinal fluid revealed pleocytosis with normal glucose and protein levels in 12 cases (86%). Rickettsial serology was performed for 15 patients. Antibody titers against *Rickettsia conorii* detected by indirect immunofluorescence are positive in only 5 patients. Cerebral CT scan is normal in 3 cases. All patients received antibiotherapy: fluoroquinolone (11 cases), doxycycline (5 cases) for a mean duration of 13 days. Mechanical ventilation was initiated for 1 patient. Clinical outcome is favourable for all patients.
Conclusion: Rickettsial infection should be considered as a cause of neurological involvement in endemic areas. Early diagnosis and appropriate treatment are mandatory to prevent fatal issue of this potential complication of Mediterranean spotted fever.

Molecular studies with fungal pathogens

P724 Use of a new PCR to identify *Candida* species isolated directly from a positive blood culture

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Objective: Evaluation of a multiplex real-time PCR to identify *Candida* species from positive blood cultures.
Materials and Methods: A PCR technique to identify *Candida* was evaluated using: a) the fungus-specific universal primer pair, ITS3 and ITS4, to amplify a rDNA region (a portion of the 5.8S region, the ITSII region and a portion of the 28S region); b) five species-specific TaqMan probes to identify the most frequent species isolated in clinical samples (*C. albicans*, *C. parapsilosis*, *C. glabrata*, *C. tropicalis* and *C. krusei*); and c) a generic *Candida* TaqMan probe. The PCR was used on the supernatant of positive blood cultures shown to contain yeasts by Gram staining. DNA extraction was made using the EZ1 automatic system (Blood DNA protocol, Quiagen). For the PCR reaction, 5 μl from the extraction were transferred to a tube containing *C. albicans*, *C. parapsilosis* and *C. glabrata* species-specific TaqMan probes; if negative, the procedure was repeated with *C. tropicalis* and *C. krusei* species-specific TaqMan probes. Finally, if there was no amplification, the reaction was repeated with the generic *Candida* TaqMan probe.
Results: 73 blood cultures from 63 patients (32 males/31 females; 51 adults/12 children) were used. Patients were admitted to ICU (43%), surgery (28.5%), haematology and oncology (8%) and internal medicine wards (20.6%). Positive blood cultures were subcultured on blood agar, Sabouraud agar and CHROMagar *Candida*. Subcultures resulted in: 61 pure cultures, 12 mixed (yeast and bacteria) and 1 mixed to two *Candida* species. The species identified from different cultures were: 29 *C. albicans*, 19 *C. parapsilosis*, 18 *C. glabrata*, 6 *C. tropicalis*, 1 *C. lipolytica* and 1 *C. guilliermondii*. The first PCR (*C. albicans*, *C. parapsilosis* and *C. glabrata* species-specific TaqMan probes) correctly identified all *C. albicans* and *C. parapsilosis*, and 16 out of 18 *C. glabrata*. The second PCR identified all 6 *C. tropicalis*. The universal *Candida* PCR detected the remaining two species (*C. lipolytica* and *C. guilliermondii*). This PCR detected and identified, directly from blood culture, 94.6% of yeast causing fungaemia.
Conclusion: The proven real-time PCR permits the detection and identification of species causing candidaemia in less than three hours, while the conventional systems take at least 48 hours.

P725 Identification of *Candida* species by restriction enzyme analysis

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Background: Identification of *Candida* species isolated from clinical specimens gives information about the antifungal susceptibility as well as sheds light to the choice of empirical treatment.

Objectives: This study was planned to apply restriction enzyme analysis (REA) for more rapid and reliable identification of *Candida albicans* and non *Candida albicans* species which had been previously identified by conventional methods and to compare the results.

Methods: In this study 146 *Candida* strains (40 *C. albicans*, 27 *C. parapsilosis*, 26 *C. tropicalis*, 25 *C. glabrata*, 11 *C. kefyr*, 10 *C. krusei*, and seven *C. guilliermondii*) isolated from various clinical specimens and *C. albicans* ATCC 14053, *C. parapsilosis* ATCC 90018 and *C. krusei* ATCC 6258 were included. The strains were identified according to germ tube test, morphology at cornmeal tween 80 agar and CHROMagar *Candida* and API 20C AUX system.

PCR was performed by using primers which targeted ITS1, 5.8S rDNA and ITS2 regions. PCR products were digested with MwoI enzyme for all species and with BslI for *C. parapsilosis* and *C. tropicalis* strains. Sequence analysis of four *C. albicans* and two *C. guilliermondii* isolates which resulted in different restriction patterns was performed.

Results: Calculated lengths of REA products obtained by MwoI enzyme are presented in the table. Restriction of *C. parapsilosis* and *C. tropicalis* isolates with BslI resulted in three bands of 413, 94 and 63 bp and three bands of 326, 187 and 63 bp, respectively. Three *C. albicans* isolates which produced a different restriction pattern had point mutations (guanine instead of adenine); one isolate had insertion/deletion type mutation and these mutations were the reason of a different pattern. In addition, sequence analysis of two *C. guilliermondii* isolates showing various fragments with different lengths were 66.6% similar with GenBank *C. guilliermondii* var. *guilliermondii* and 91.2% similar with *C. guilliermondii* var *membranaefaciens* (nucleotide seqeunce). Because of this high nucleotide sequence similarity, these isolates were accepted as *C. guilliermondii* var *membranaefaciens*.

Lengths of REA products, bp (no. of isolates)

C. albicans	*C. parapsilosis*	*C. tropicalis*	*C. glabrata*	*C. kefyr*	*C. krusei*	*C. guilliermondii*
141,84,261 (36)	336,146,88 (27)	325,154,97 (26)	414,174,171,86,80 (25)	370,430 (11)	289,134,83,54 (10)	355,302 (5)
184,141,165,95 (4)						390,300 (2)

Conclusion: As a result, we can conclude that restriction enzyme analysis with MwoI and BslI enzymes can be used for the identification of *Candida* species which need rapid identification or which are problematic with conventional methods that are still accepted as gold standard although some variations can be observed with this method.

P726 Nucleotide differences in internal transcribed spacer regions of rDNA and 5-flucytosine resistance among clinical *Candida dubliniensis* isolates in Kuwait

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Objective: Clinical *Candida dubliniensis* isolates are classified into four genotypes based on nucleotide sequences of internal transcribed spacer (ITS)1 and ITS2 regions of rDNA. Some of the isolates from the Middle Eastern region are resistant to 5-flucytosine. This study was carried out to determine nucleotide differences in ITS region (including ITS1–5.8S rRNA-ITS2) of rDNA that define 5-flucytosine resistance among clinical *C. dubliniensis* isolates in Kuwait.

Methods: A total of 76 *C. dubliniensis* isolates recovered from various clinical specimens in Kuwait were assigned to one of four genotypes based on amplification of rDNA with genotype-specific primers. The susceptibility of the isolates to commonly used antifungal agents including 5-flucytosine was performed by Etest. DNA sequencing was performed to correlate specific nucleotides in ITS region of rDNA with the four genotypes and to define signature residues associated with resistance of *C. dubliniensis* isolates to 5-flucytosine.

Results: Majority (49 of 76, 64%) of *C. dubliniensis* isolates belonged to genotype 1, DNA sequences of ITS region of rDNA from 12 randomly selected isolates were identical and matched completely with the prototype strain CD36. A total of 21 isolates (21 of 76, 28%) yielded an amplicon with genotype 4 specific primers only and their ITS region sequences matched completely with the prototype strain of genotype 4. Only 1 isolate belonged to genotype 3. None of the *C. dubliniensis* strains belonged to genotype 2. Five isolates yielded amplicons with more than one genotype-specific primer pair and DNA sequences of the ITS region from these isolates identified them as hybrid strains. A total of 21 *C. dubliniensis* isolates were resistant to 5-flucytosine, all 21 belonged to genotype 4 and were characterised by the presence of a T residue at nucleotide position 82 in the ITS region.

Conclusions: Only three of four genotypes based on ITS region sequences were detected among clinical *C. dubliniensis* isolates in Kuwait with most (64%) of the isolates belonging to genotype 1. All *C. dubliniensis* isolates resistant to 5-flucytosine belonged to genotype 4 and were defined by a signature nucleotide in the ITS region of rDNA. The *C. dubliniensis* hybrid strains closely related to genotype 4 but not containing this signature nucleotide were not resistant to 5-flucytosine.

P727 Genotypic relatedness of *Candida* spp. in neonatal intensive care unit

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Objectives: We aimed to characterise the genetic relatedness of *Candida* species isolated from samples taken on clinical grounds in two NICUs in Estonia using pulsed field gel electroforesis (PFGE).

Methods: *Candida* spp. were isolated from August 2005 to May 2006 in unit A and from June 1999 to November 2005 in unit B and stored in sterile water or cryopreserved. The species were identified with CHROMagar *Candida*, Corn Meal Agar with Tween 80 and API *Candida*. The relatedness of the isolates was analysed by optimisation of previously described PFGE method as follows: BssHII digested DNA was electrophoresed with a voltage of 4V/cm, temperature 14 degrees C, linear ramped pulse times 5 to 50 seconds and run time of 20 hours.

Results: A total of 83 isolates (12 from blood, 57 from trachea and 14 from other sites) from 52 neonates were analysed. Of 16 invasive isolates *C. parapsilosis* (n = 8) and *C. albicans* (n = 6) were most commonly seen. *C. guilliermondii* (30%), *C. parapsilosis* (30%) and *C. albicans* (29%) were predominant isolates in colonised patients. In unit A all 6 *C. albicans* isolates were genotypically different whereas in unit B only 2 clones among 18 isolates were identified. Of 25 isolates of *C. guilliermondii* and *C. parapsilosis* each only 2 and 3 different clones, respectively, were seen. However, *Candida* strains isolated in 2000–01 differed from those obtained in 2005–06 by one or two bands suggesting that microevlolutionary changes within a strain had occurred.

Conclusion: Although *C. guillliermondii* was a frequent coloniser in the NICU patients, yet it was rarely associated with the invasive disease. The presence of a limited number of different clones of *C. guilliermondii* and *C. parapsilosis* indicates permanent source in the NICU and suggests for transmission by direct or indirect contact and cross-colonisation by healthcare workers.

P728 Establishing a rep-PCR DNA fingerprinting library for moulds

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Objectives: Discrimination among moulds is often difficult. The advances in many areas in microbiology are limited when looking at moulds. Morphololgical characterisation and staining techniques are still very common. Sequencing and real-time PCR can be used

but additional technologies are needed for investigation of moulds. Previous studies have shown the promise for the use if rep-PCR DNA fingerprinting as a molecular method to characterise some moulds. This study reports the compilation of an expanded Mould database as an additional tool that can be applied to mould characterisation and subspecies discrimination.

Methods: A total of 250 previously characterised moulds (including strains from 30 Genus/species) obtained from environmental and clinical sites and ATCC reference strains were cultured. The isolates were extracted and then amplified using the Mold Kit for DNA fingerprinting. The amplified product was separated using a microfluidic based detection. The resulting data was analysed using dendrogram analysis and a Mould library was created using the DiversiLab Software. An additional 50 mould isolates were processed and compared to the library for validation.

Results: The resulting fingerprints from the first 250 characterised isolates grouped into multiple clusters. No different Genus/species showed the same fingerprint pattern. Some species had more than one fingerprint pattern, or cluster, indicating discrimination to the subspecies and/or strain level. Many of the 50 isolates used for validation showed matches to the library. Some isolates formed new clusters but no false positive groupings are seen.

Conclusions: These data suggest that a characterised library may prove useful to provide additional information or confirmation when using DNA fingerprinting for moulds. The DiversiLab System shows promise as a tool to aid in surveillance and identification of hospital acquired infections and epidemiological studies.

P729 Evaluation of differential expression of calnexin gene during transition from mycelial to the yeast infective of the fungus *Paracoccidioides brasiliensis* and localisation of native protein

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Introduction: *Paracoccidioides brasiliensis* is a dimorphic fungus responsible for paracoccidioidomycosis (PCM); man is infected by inhalation of conidia. The cellular differentiation of *P. brasiliensis* from mycelium (M) to yeast (Y) in the lungs is essential factor to establish the infection. Calnexin is a chaperone that recognises specifically monoglucosylated glycoproteins in the endoplasmic reticulum, being an essential component of the folding process of nascent secreted glycoproteins. Objective: Evaluation of differential expression of the PbCNX transcripts and analysis of cellular localisation of protein native.

Methods: RNA isolated from *P. brasiliensis* after heat shock and at different phases. The cDNA encoding PbCnx was cloned and overexpressed as recombinant protein in *Escherichia coli* and used to immunise rabbits in order to obtain anti-PbCnx serum.

Results: Expression of the PbCNX transcripts was investigated in total RNA isolated from *P. brasiliensis* after heat shock and at different phases of the mycelium to yeast (M-Y) transition or yeast and mycelium phases (Y and M), by real time RT-PCR. Our results suggest that there was an increased in PbCNX expression at 10 h after temperature change (25 to 36ºC), but normal levels was observed at the last point analysed (M-Y). Additionally, there was a 3.5-fold increase in transcription after heat shock at 42ºC for 60 min. Antibodies used in the reactions recognise specifically a single 70 kDa in the cell wall (alkaline-mercaptoethanol extract) component, compatible with the predicted size of the protein.

Conclusion: The evaluation of differential expression during transition from mycelial to the yeast infective of calnexin suggest that is a heat shock protein and is the first time that PbCnx has been observed on the cell surface, however, its function is speculative. Financial support: FAPESP and CNPq.

P730 Molecular karyotype of Sporothrix schenckii: analysis of chromosomal polymorphism among different isolates

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Introduction: Classical genetic analysis of S. schenckii, aetiological agent of sporotrichosis, has been impaired by the absence of known teleomorphic stage. Experiments employing in vitro mutagenesis led to the selection of few mutants with stable phenotypes, since the number of revertants were high. The genetic composition of the fungus is poorly known and information about the genome size and chromosome organisation is scarce.

Objective: This study aims to provide a better characterisation of S. schenckii chromosomal polymorphisms among human clinical isolates (lymphocutaneous forms), and ambient sources.

Methods: Approximately 2X107 yeast cells were imbibed in low melting agarose. Spheroplasts were obtained by digestion of the cell walls with zymolmyase and disrupted in a solution containing EDTA, sarcozyl and proteinase K. Electrophoretic separation was performed under pulsed-field gel electrophoresis (PFGE) conditions.

Results: Differences in electrophoretic mobility of bands suggest the existence of chromosomal polymorphism and make it difficult to correlate the banding pattern among isolates. The analysis of our results shows 5 to 6 chromosomal bands and genome size about 28 Mb. Differences among the number of chromosomes and electrophoretic mobility of chromosomal bands were observed. New and distinct profiles emerged when comparing our data with previously described ones. The results also indicated that isolates from different geographical areas present quite similar banding patterns.

Conclusion: The results were consistent with a haploid number of 4–6 chromosomes. The haploid genome was estimated to be in the range of 28 Mb. The plasticity fungus genome could have implications for maintenance of genome functionality and for the control of gene expression.

Financial support: FAPESP and CNPq.

P731 Characterisation and expression of the gene encoding a gli-coprotein of 70 kda (gp70) from *Paracoccidioides brasiliensis*

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Introduction and Objectives: *Paracoccidioides brasiliensis* is a thermodimorphic fungus that causes Paracocciodioidomycosis (PCM), human systemic granulomatous disease prevalent in Latin America. Most of the genes encoding and proteic sequences of relevant fungal antigens remain uncharacterised. *P. brasiliensis* produces some important antigens like gp43 which has been fully characterised, and a 70 kDa glycoprotein (gp70) recognised by 96% PCM patients'sera. It has been shown that this purified antigen modulates murine peritoneal macrophages functions and that passive immunisation of mice with specific anti-gp70 mAbs before fungal infection, significantly inhibited lung granuloma formation. The purpose of this work was molecular cloning and characterisation of the gene that encodes gp70 from *P. brasiliensis* followed by heterologous expression of the recombinant antigen to better understand the role of the gp70 in PCM.

Methods and Results: Using degenerated oligonucleotides corresponding to peptides obtained by microsequencing from gp70, recognised and isolated with specific mAB (C5F11), a 384-bp DNA fragment was amplified from the *P. brasiliensis* genomic DNA (pbgp70). This fragment showed 97% of similarity with one of the Expressed Sequence Tags (ESTs) in *P. brasiliensis* EST databank. Specifc oligonucleotides were synthesised attempting to obtain the sequence and amplifying the genomic and cDNA sequences corresponding to this EST. Using these specific oligonucleotides, 2.0 kb products were obtained by PCR of the genomic DNA and cDNA. These fragments were cloned and sequenced by primer walking. In preliminary sequence analysis it was found the open reading frame in a 1.6 kb fragment. Deduced amino acid sequence showed prevalence of hydrophilic regions which probably contains B-cell epitopes, one putative T-cell mouse epitope and high Jameson-Wolf Antigenic Index. BLAST analysis showed homology with putative proteins from the fungus *Coccidioides immitis*, *Aspergillus fumigatus*, *Ajellomyces capsulatus*, which are phylogenetically close to *P. brasiliensis*. Other oligonucleotides are being synthesised to achieve the heterologous expression of the cDNA sequence and subsequent obtention of the recombinant protein for further biological assays.

Conclusion: The 2.0 kb genomic DNA and cDNA cloned sequences presents some predicted characteristics of the *P. brasiliensis* native gp70 and may represent the gene of the gp70 antigen. Financial Support: CAPES

P732 Identification of *Paracoccidioides brasiliensis* highly virulent isolate ligands peptides by phage display with fungicidal activity in vitro

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Introduction: Paracoccidioidomycosis is a human systemic granulomatous disease, prevalent in South America, caused by a thermodimorphic fungus, *Paracoccidioides brasiliensis*. This fungus presents complex antigenic structure and some of these components have been related with its pathogenicity, of which little is known. Objective: To isolate molecules expressed in the surface of highly virulent but not in non-virulent isolated yeast cells of *P. brasiliensis* by phage display techonology, thus identifing possible virulence factors.
Methods: A phage display library containing the insert CX7C was screened to search high-virulent isolate of yeast cells of *P. brasiliensis* ligands by Biopanning and Rapid Analysis of Selective Interactive Ligands method. After three rounds of selection, the DNA corresponding to peptide inserts of randomly chosen phage clones were sequenced. Peptide sequences were then analysed according to their enrichment and by Clustal W sequence alignment. Selected motifs were used to search nonredundant protein databanks (National Center for Biotechnology Information [NCBI] BLAST.
Result: The B10.A mice were inoculated with yeasts of different isolates of *P. brasiliensis*. Animals that received non-virulent isolates Pb18 and Pb265 groups behaved as the control group which received only PBS, but the group that was infected with highly virulent isolate Pb18 died 90 days after injection. We identified three peptides (CGSYGFNAC, CGLRLESTC and CGLRLESTC) that bound to the surface of the high-virulent but did not or did less in non-virulent isolate of *P. brasiliensis* in all experimental conditions tested ($P < 0.01$). Other experiment showed that phage-cells binding was dose-dependent. By EIA assay with synthetic peptides, a significant binding of these peptides to gp43 (the majority molecule) was not observed. These peptides exhibited fungicidal activity in vitro against *P. brasiliensis* by inhibition of colony forming units.
Conclusion: The peptides CGSYGFNAC, CGLRLESTC and CGLRLESTC may represent ligands of molecules that can be involved in the virulence and pathogenicity of this fungus. Financial support: FAPESP.

P733 Genotyping of *Trichosporon asahii* strains isolated from urinary tract infections in a Turkish university hospital

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Objectives: *Trichosporon* infections are associated with a wide spectrum of clinical manifestations ranging from superficial cutaneous involvement to severe systemic disease depending on the immune status of the patient. *Trichosporon asahii* appears to be the most important species in the genus. Invasive infections with *T. asahii* has been described most frequently in immunocompromised hosts, although some case reports of *T. asahii* infections in immuncompetent patients exist. In this study, we evaluated the genotypic relatedness of *T. asahii* strains isolated from non-immunocompromised patients' urinary tract infections.
Methods: A total of 25 *T. asahii* urinary strains were studied. The isolates were obtained from17 immuncompetent patients (11 male, 6 female, median age 44) hospitalised at different wards (neurosurgery, urology, nephrology, orthopaedics, reanimation) of Ankara University Ibn-i Sina Hospital between July 2004-April 2005. None of the patients were neutropenic during the period when *T. asahii* was isolated. *T. asahii* identification was made by using the API 20C AUX (BioMérieux Vitek, USA) and confirmed by PCR by using genus and species specific primers. Genotyping of *T. asahii* strains were made by RAPD and REP-PCR methods by using M13 and ERIC2 primers, respectively.
Results: All of the isolates gave amplicons by *Trichosporon* specific primers. When species specific PCR was performed, 23 of the isolates obtained from 15 patients gave amplicons. These 23 isolates were subjected to genotyping. RAPD analysis by M13 primer resulted in clustering of the isolates into 5 groups; four of the groups were composed of single isolate, and the fifth group contained 19 isolates. The results of REP-PCR analysis showed good corelations with the data obtained by RAPD method, although this method produced three different groups. Two of the groups contained single isolates which corresponded to the two groups of RAPD analysis. The third group consisted of 21 isolates containing the two isolates which were distributed to different groups by RAPD analysis.
Conclusion: Here we report 15 immuncompetent patients with urinary tract infections due to *Trichosporon asahii*. In this first study of invasive trichosporonosis from Turkey, 19 of the 23 isolates were found to be genotypically related, suggesting that a single genotype was prevalent in the hospital environment. The genotypically unrelated four isolates were probably of endogenous origin.

P734 Clinical performance of FXG^{TM} : RESP (Asp +) assay for aspergillus on respiratory specimens

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Objectives: Early diagnosis of invasive aspergillosis (IA) is essential to survival. Classical microbiological methods are slow and insensitive. Real-Time PCR offers the prospect of both faster and more sensitive microbiological confirmation of IA. FXG : RESP (Asp +) is a new test kit that detects both *Aspergillus* spp. and *Pneumocystis jirovecii*, utilising molecular beacons. This ready to use PCR assay consists of an extraction kit plus PCR assay and is CE marked. In this report we focus on the clinical performance for *Aspergillus* spp.
Methods: The FXG : RESP (Asp +) real-time PCR kit was tested on 198 respiratory specimens from a wide variety of patient groups, collected from 4 European hospitals. Analysis was carried out using an AB7500 thermocycler. Samples of BAL were mostly stored at –20 to –80ºC prior to DNA extraction using the MycXtra^{TM} fungal DNA extraction kit. Some sputum samples were also processed. The FXG : RESP (Asp +) results were compared with culture for *Aspergillus* spp. The whole assay time including extraction is <4 hours from BAL and <5 hours from sputum. The limit of detection was ~1 genome for *A. fumigatus* (as assessed by purified Af293 DNA), and this was used as the clinical cut-off. An internal amplification control reaction within the kit detects inhibitors that might affect the PCR reaction, although positive results in the presence of inhibition may be reported.
Results: Overall, for all 198 respiratory samples the sensitivity was 74% and specificity 93% with positive and negative predictive values 76% and 92% respectively. On 75 freshly collected sputum and other lower respiratory tract samples the sensitivity and specificity were 79% and 88% respectively. The FXG : RESP (Asp +) assay also detects *Penicillium* spp. as the target sequence in this fungus is identical. 3 samples were culture positive for *Penicillium* spp., and positive for FXG : RESP (Asp +) and recorded as 'false positives'. The FXG : RESP (Asp +) does not detect Zygomycetes or *Candida* spp., and this was confirmed in the clinical study.
Conclusions: Overall the speed of detection and sensitivity of the FXG : RESP (Asp +) assay will bring considerable clinical benefits. Additional prospective and supportive clinical trials are ongoing.

P735 **Identification of the pre-mRNA splicing protein U2AF35 in the opportunistic fungus *Pneumocystis carinii***

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The opportunistic fungus *Pneumocystis carinii* (PC) is an intractable organism responsible for severe pneumonia in patients with AIDS or other immunosuppressive conditions. PC is phylogenetically similar to the non-pathogenic yeasts *S. cerevisiae* and *S. pombe*. The regulation of gene function is a complex process critical to the survival of eukaryotes, and thus presents potential targets for novel therapy. Pre-mRNA processing with removal of intervening sequences (introns) is an important step in gene regulation. The assembly of multiple proteins on small nuclear RNA occurs in the spliceosome. Although many components of this molecular machinery is conserved in eukaryotes, there are many significant differences between *S. cerevisiae*, *S. pombe* and mammals. The process of gene regulation in PC is unknown. We have previously reported that PC genes contain multiple small introns, yet lack features found in *S. cerevisiae* and *S. pombe* (Infection and Immunity, Nov. 1999, p. 6157–6160). Here we report the identification and cloning of the PCU2AF35 gene, encoding a critical splicosomal protein required for processing the 3′ end of the intron. PCU2AF35 is a 621-bp ORF encoding a predicted 206 amino acid protein. The predicted molecular weight is 24.1 kDa, similar to *S. pombe* U2AF35. PCU2AF35 contains a conserved RNA recognition motif (RRM) postulated to bind RNA sequences. PCU2AF35 is most homologous to *S. pombe* U2AF35 (72% identity). We have expressed this gene as a recombinant protein to determine its function in PC intron removal. Our investigations may provide new insights into the pathogenesis of PC and potential new therapeutics for treating PC pneumonia through identification of mechanisms involved in PC life cycle regulation.
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P736 **Rapid identification and differentiation of Trichophyton species, based on sequence polymorphisms of the ribosomal internal transcribed spacer regions, using rolling circle amplification**

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Objectives: To develop a novel padlock probe and rolling circle amplification (RCA)-based method, for identification of single nucleotide polymorphisms (SNPs) to differentiate between major members of the genus Trichophyton.
Methods: The ITS region (ITS1, 5.8S and ITS2) of 42 dermatophytes belonging to seven species (Trichophyton rubrum, T. mentagrophytes, T. soudanense, T. tonsurans, Epidermophyton floccosum, Microsporum canis and M. gypseum) were sequenced. Polymorphisms were identified after multiple sequence alignment and comparison with relevant archived GenBank sequences. Either species-specific or group-specific padlock probes were designed to detect the species above.
Results: DNA sequencing demonstrated demonstrated intra-species genetic variation for T. tonsurans, T. mentagrophytes and T. soudanense, but not for T. rubrum. Signature sets of SNPs between T. rubrum and T. soudanense (4 bp difference), and T. violaceum and T. soudanense (3 bp difference) were identified. The RCA assay correctly identified five Trichophyton species. Although use of two "group-specific" probes targeting both the ITS1 and ITS2 regions were required to identify T. soudanense, the other species were identified by single ITS1-, or ITS2-targeted species-specific probes. There was good agreement between ITS sequencing and RCA assay.
Conclusion: Despite limited ITS sequence-based variation between Trichophyton spp., the RCA-based SNP detection assay showed potential as a simple, specific and reproducible method for rapid (2 hours) identification of Trichophyton spp.

P737 **Genotypes of human *Blastocystis hominis* isolates and its pathophysiological varaibility in exeperimentally infected rats**

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Background: A definite correlation between the distinct genetic variability present in *Blastocystis hominis* parasite and its pathogenecity has not yet been confirmed.
Objectives and Methods: The first objective of this study was identify the genotypes of *B. hominis* clinical isolates obtained from 28 gastrointestinal symptomatic patients and 16 asymptomatic individuals by PCR using sequenced-tagged site (STS) primers. The second objective was evaluation the pathophysiological variability between different *B. hominis* genotypes induced in experimentally infected rats by identify the histopathological changes, the mortality rate and the intestinal cell permeability in Ussing chamber. Infected rats with asymptomatic and symptomatic human isolates were used.
Results: Only 4 *B. hominis* genotypes were identified among all the human clinical isolates. Subtypes 1, 2, 3 and 4 were detected among 18.2%, 9.1%, 54.5% and 18.2% of the human isolates. In symptomatic isolates subtypes 1, 3 and 4 were detected in 28.6%, 57.1% and 14.3% respectively. In asymptomatic isolates subtypes 2, 3 and 4 were identified in 25%, 50%, and 25% respectively. Subtype 3 was the commonest among humans. Different degrees of pathological changes were found among infected rats by symptomatic subtypes as compared with asymptomatic subtypes. The moderate and severe degrees of pathological changes were found only in symptomatic subtypes infected rats while mild degree was found only in asymptomatic subtypes infected rats. No extra intestinal lesions were found. Only subtype1 induced mortality rate with 25% among infected rats. On evaluation of the intestinal cell permeability, a prominent increasing in short circuit current (ÄIsc) was found in symptomatic subtypes 1 compared to symptomatic subtypes 3 and 4 infected rats. Meanwhile, minimal effects were found in asymptomatic and control groups.
Conclusions: Subtype 1 is relevant to the pathogenecity of *B. hominis* while, subtype 2 is irrelevant. The presence of subtypes 3 and 4 among symptomatic and asymptomatic isolates suggested that the variability on Blastocystis pathogenecity among these subtypes may be related to the presence of sub-strains (pathogenic or non-pathogenic) which needs further studies.

Amoeba

P738 **Real-time PCR as a semi-quantitative alternative for microscopic detection of *Dientamoeba fragilis***

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Objectives: Since the discovery of *Dientamoeba fragilis*, the pathogenicity of this organism has remained controversial. Microscopic detection did not imply a quantification of this frequently found faecal parasite. A new real-time PCR based on the 5.8S ribosomal RNA gene was developed for the detection of *D. fragilis* in faecal samples. We examined if this could provide a semi-quantitative alternative for microscopic detection.
Methods: Microscopic examination was carried out on IHK stained faecal samples collected after SAF fixation with the Triple Faeces Test (TFT). A selection of 200 positive and 200 negative samples was made. Real-time PCR was performed on the corresponding fresh stool samples. The PCR also included an internal control (PhHV) to detect inhibition of the amplification by faecal constituents in the sample.
Results: In the first half year of 2007, 2052 faecal samples were examined of which 394 were positive for *D. fragilis* (19.2%). In the selection of 400 samples no inhibition of amplification was found. In 200 microscopically negative stool samples *D. fragilis* was amplified in 12 cases. In these 12 samples, the presence of *D. fragilis* could be confirmed microscopically after re-analyses of the permanent staining

preparations in 9 cases, corresponding to a specificity of 98.5%. In a selected group of 200 faecal samples from patients in which one or two preserved samples showed *D. fragilis* trophozoites, *D. fragilis*-specific amplification was detected in 184 DNA samples wich corresponds to a sensitivity of 92%. The mean Cp value of the positive samples was 30.7 with a range from 20.1 to 37.8.

Conclusions: The real-time PCR for the detection of *D. fragilis* achieved 98.5% specificity and 92% sensitivity. The use of this assay in a diagnostic laboratory offers the possibility of introducing DNA detection as a feasible technique for the routine diagnosis of intestinal *D. fragilis* infections and provides a reliable semi-quantitative measure for further pathogenicity studies.

P739 Molecular assays to diagnose *Entamoeba histolytica* and *E. dispar* infections in a non-endemic area (Parma, Italy)

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Objectives: The detection of *Entamoeba histolytica* (Eh), causative agent of amoebiasis, is an important goal of the clinical parasitology laboratory. The identification of *E. dispar* (Ed) as a morphologically identical but non-pathogenic species has highlighted the need for non-microscopic detection methods able to differentiate the 2 species. Moreover, a reassessment of the worldwide prevalence of amoebic infection based on discriminatory methods has been strongly recommended by WHO.

The area in which our laboratory is located has seen in recent years an increase of people travelling through and a significant flow of immigration from tropical countries which has led to a parallel increase in imported infections.

Thus, the aim of this study was to promptly diagnose and discriminate Eh and Ed infections and to assess their occurrence among patients with suspected intestinal parasitosis presenting to the University Hospital of Parma.

Methods: The specimens analysed in this study were selected from the whole of the clinical samples sent to our laboratory with the suspicion of intestinal parasitosis during 2003–2007 and subjected to standard procedures (microscopic examination) for the detection of intestinal parasites. In particular, 730 samples (715 faeces, 7 liver abscess samples, 8 intestinal biopsies) belonging to 407 patients were analysed. The samples were subjected to culture for intestinal protozoa and molecular assays (conventional or Real-Time PCR) able to distinguish Eh from Ed DNA.

Results: Eight patients (1.96%) proved to be infected by Eh (3 with extraintestinal and 5 with intestinal amoebiasis; 5 with imported amoebiasis and 3 with the disease acquired in Italy) and 36 by Ed (8.84%). In particular, in 4 of the 8 patients with Eh infection, only molecular methods were able to detect the presence of the parasite.

Conclusion: Our results underline that traditional methods underestimate the prevalence of amoebic infection and highlight the important diagnostic role that PCR can play. Species differentiation by PCR becomes an important tool for clinicians as it permits them to focus on Eh infections and to avoid treatment when non-pathogenic Ed is present. The relatively high prevalence of Ed infections observed in this study versus the more rare Eh infections, is indicative of the existence in the community of a faecal-oral route of agents with an exclusively inter-human circulation and calls for the activation of adequate control measures.

P740 Amoebic keratitis in Iran (1997–2007)

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Objective: Amoebic keratitis introduced as a painful corneal infection which sometimes lead to poor vision and blindness. The main goal of this study was to report amoebic keratitis during ten years from 1376–1386 in patients who was suspected to amoebic keratitis and referred to parasitology laboratory, School of Public Health, Tehran Medical University, Iran. Other aim was to assess the major risk factor for developing this sight-threatening disease. Comparison of lens culture and corneal scrapes culture also performed.

Methods: During 1376–1386, 142 patients referred to department of parasitology, School of Public Health, Tehran Medical University. Details of each patient such as age, sex, history of contact lens wear, type of contact lens, clinical symptoms were record in questioners. Keratitis was diagnosed on the basis of culture of lenses and/or corneal scrapes on non-nutrient agar overlaid with *Escherichia coli* and direct microscopy of lenses and/or corneal scrapes.

Results: Among 142 patient who referred to department of parasitology, School of public health, Tehran Medical University, Iran, 49 patients (34.5%) had Amoebic keratitis. 73.46% of these patients were from Tehran but there were a few cases from other cities. The commonest age was between 15–25 (75.5%) and more female (37:12) were identified then male. It is worth to mention that 44 patients (89.79%) were contact lens wearers which among them 41 patients (93.18%) wore soft contact lens and only three patients suffer from amebic keratitis because of wearing hard contact lens. Other finding of this study demonstrated that the most common sign of the patients was severe pain combined with photophobia.

Conclusion: This study indicate that Acanthamoeba keratitis continue to rise in Iran. This is due to increase frequency of lens wearers as well as consideration of ophthalmologist to Acanthamoeba as an agent of keratitis and improvement of laboratory methods. Another finding of this research was the confirmation of soft contact lens as a major risk factor. It is recommended to educate contact lens wearers for regular disinfection. Besides, culture of corneal scrapes was negative in most of cases, so lens culture were performed which had a much better result.

P741 Diagnosis of dientamoebiasis: efficacy of culture and comparison of three culture media

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Objectives: The diagnosis of dientamoebiasis depends on the demonstration of *Dientamoeba fragilis*, a common but rarely identified intestinal protozoon. Its nuclear structure can only be seen with permanent stains, such as trichrome or iron hematoxylin; therefore, examination of permanent stained smears of fresh or fixed stool samples by an experienced microscopist is obligatory for the diagnosis of dientamoebiasis. Culture is known to be a more sensitive method but it is out of scope of many laboratories in routine diagnosis. However, it may identify the overlooked cases with microscopy and is reported to be less laborious than trichrome staining. Our aim was both to assess the efficacy of culture and compare the efficacies of three culture media, Robinson's, Dobell's and Talis's diphasic egg medium (TDEM) in the diagnosis of dientamoebiasis.

Methods: Among the 93 dientamoebiasis patients diagnosed in the Parasitology Laboratory of Celal Bayar University Hospital, stool samples of 80 and 75 patients were inoculated in Robinson's and Dobell's media, respectively, while 65 were inoculated in TDEM; stool samples of 61 patients were inoculated in all three media. In addition, stool samples of 160 Dientamoeba-negative individuals diagnosed with the microscopic examination of trichrome stained smears were inoculated in all three media. All culture tubes were checked by the microscopic examination of a drop of sediment at 48, 72 and 96 hours after inoculation. The sensitivity and specificity values of Dobell's medium and TDEM were assessed compared to Robinson's medium.

Results: Diagnostic efficacies of each medium were found to be 90%, 76% and 86% for Robinson's, Dobell's and TDEM, respectively. Among the 160 microscopically-negative samples, the growth of *D. fragilis* was detected in 17 (11%), 5 (3%) and 6 (4%) in Robinson's, Dobell's and TDEM, respectively. All positive samples in Dobell's medium and TDEM were also found to be positive in Robinson's medium.

Conclusion: Our results suggest that microscopy may overlook significant number of dientamoebiasis cases, which are detectable with culture, and Robinson's medium may be used effectively even in routine diagnosis of dientamoebiasis, instead of trichrome staining. Culture is by far less expensive than molecular methods and may be preferred where

novel diagnostic methods, such as PCR, are not available due to their higher costs, especially in developing countries.

P742 Detection of amebiasis among men who have sex with men, with or without human immunodeficiency virus infection

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Objectives: Men who have sex with men (MSM), regardless of HIV status, are at increased risk for amebiasis because of oral-anal sexual contact in Taiwan. In this study, we aimed to evaluate the association of seroconversion for *Entamoeba histolytica* infection with newly acquired intestinal infection with *E. histolytica* among MSM.
Methods: Between 1 January, 2007 and 30 November, 2007, 143 MSM (128 HIV-infected) who were free of gastrointestinal symptoms provided both blood and stool specimens for serological tests and specific amebic antigen tests for *E. histolytica* infection, respectively. Anti-*E. histolytica* antibodies were determined using indirect hemagglutination (IHA) assay (Cellognostics, Boehhringer Diagnostics GmbH, Marburg, Germany), while stool amebic antigen was determined using commercial test kits (ENTAMOEBA TEST, TechLab, Branchburg, NJ) followed by polymerase chain reactions (PCR) using specific primers for *E. histolytica*.
Results: In this survey, the prevalence of intestinal E. histolytca infection was 9.1% (13/143) and the seroprevalence of *E. histolytica* infection (IHA titers $\leqslant$32) was 17.5% (25/143) among the subjects. MSM who were seropositive were at statistically significantly higher risk for intestinal *E. histolytica* infection with an odds ratio of 25.56 (95% confidence interval, 6.316, 103.4) ($p<0.0001$).
Conclusion: We found that MSM who were IHA-seropositive for E. histolytcia was more likely to present with intestinal *E. histolytica* infection than those who were seronegative. In clinical settings where amebiasis is suspected and stool samples may not be readily available, serologies for *E. histolytica* is a cost-effective screening alternative to identify *E. histolytica* infection among MSM.

P743 Indigenous amebiasis in hypoendemic area: myth or reality?

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Amebiasis is one of the three most common causes of death from parasitic disease. Belarus is considered a non-endemic country since indigenous transmission of the *Entamoeba histolytica* parasite may be virtually non-existent. However, invasive intestinal and extra-intestinal infections have also been found among Belarusian population.
Objectives and Methods: The medical records of in-patients, who had been diagnosed as cases of amebiasis, at some hospitals in Minsk in 1994–2006, were retrospectively reviewed. This grope consist of 24 patients, 13 females and 11 males, average patient age was 56.4 years (range 22–81 years). To avoid overdiagnosis in each case, amebiasis was confirmed by the identification of hematophagous trophozoites of *Entamoeba histolytica* by microscopic examination of stool, pus and tissue intravital and postmortem. Unfortunately, this is the only test available in Belarus now. According to outcomes of disease there were 16 dead patients and 8 recovered ones.
Results: The epidemic investigation shows that only 2 patients have visited endemic amebic countries. All fatal cases were misdiagnosed, before the deaths following clinical diagnoses were made: liver abscess, acute gangrenous cholecystitis (2), tumour of brain, Crohn's disease (2), intestinal tuberculosis, pulmonary tuberculosis, cancer of colon (4), mesenteric thrombosis, acute anaemia, sepsis (2). Pathologic investigations reveal various amebic intestinal damages: terminal enteritis (1), ascendant colitis (1), sigmoiditis (4), total colitis (3); extraintestinal damages: liver (7), gall bladder (3), pancreas (1), lungs (2), brain (3). All of patients were burdened of premorbid conditions (diabetes mellitus, cancer, chronic diseases of lungs, GIT, heart).
In the group of survived patients, there were 6 cases of liver amebic abscess, 1 case of complicated amebic sigmoiditis and 1 case of amebic colitis with ulcerative colitis.
Conclusions: Amebiasis can be indigenous in the former hypoendemic countries and can has very severe fatal forms. It is necessary to improve clinical diagnostic and parasitological methods, to perform an antigen detection using ELISA to provide a more reliable comment on the epidemiological pattern and clinical impact of the disease.

P744 Prevalence of Entamoebae infection in Sudan

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Objective: The frequency of *E. histolytica* and *E. dispar* was studied and sociodemographic conditions associated with *E. histolytica* and *E. dispar* infection. A descriptive study was carry out in urban area of Khartoum the capital city of Sudan.
Methods: Stools samples were examined by microscopy and positive to *Entamoeba* were analysed by PCR to differentiate between *E. histolytica* and *E. dispar*.
Results: *E. histolytica* was detected in 106/196 (54.1%) and *E. dispar* in 100/196 (51%). The age groups most infected were >30 years old followed by <9 years old. *E. histolytica* was significantly associated with diarrhoea 103/196 (52.6%) and with the presence of Red blood cells in the stools 101/196 (51.5%).
Conclusion: *E. histolytica* and *E. dispar* infections are common in Khartoum, therefore we need to apply a technique help in differentiation between them in the routine diagnosis of the samples.

Malaria

P745 Comparison of three methods of real-time PCR with blood smears and antigen detection in plasmodial infection

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Objectives: In malaria, rapid and accurate diagnosis of *Plasmodium* spp. is needed for adequate and efficient treatment. Three different rapid PCR methods were compared to the routine diagnosis method.
Methods: A transversal study was conduced in our laboratories located at Montpellier and Nîmes University Hospital. Routine method associated microscopy examination of Giemsa-stained blood smears and antigen detection by Now® Malaria kit. For control samples, antigens were not routinely searched when positive diagnosis was already obtained. DNA was extracted from the previous blood samples. The three methods of real-time PCR were optimised, and the SYBR Green was used for fluorescence detection in a LightCyclerTm instrument. The PCR1 amplified a specific sequence on the *P. falciparum* Cox1 gene. The PCR2 amplified a species-specific region of the multicopy 18S rRNA present in the four plasmodium sp that infect humans. Species differentiation was obtained by melting curve analysis. The PCR3 amplified a mitochondrial DNA sequence that allowed the differentiation of *P. falciparum* from the three others species.
PCR methods were compared between each other and with the routine method. Kappa and McNemar tests were performed.
Results: On the 245 blood samples (195 diagnosis and 50 controls) collected between 2005 and 2007, 118 samples were positive for at least one of the five methods (72 [36.9%] at diagnosis, and 46 [92%] at control).
Compared to the routine method, there was no false negative, neither in PCR1 nor in PCR3 for *P. falciparum* diagnosis. It is noteworthy that microscopy alone was negative in five samples, three of them also negative in PCR2. In all *P. ovale*, *vivax* and *malariae* infections, PCR1 was negative, as expected, whereas PCR2 and PCR3 were positive and concordant (simple Kappa coefficient = 1). Globally, for first biological diagnosis, PCR3 alone (n = 103) and the association of PCR1 and 2 (n = 195) were fully concordant with routine method. As regards control samples, the three PCR methods remained more often positive than blood smears (92% vs 34%) and this for a longer period after treatment.
Conclusion: Those three rapid PCR methods showed excellent results, used solely or in association. Nevertheless, PCR did not detect additional cases of malaria potentially undiagnosed by routine testing.

P746 Epidemiology of imported malaria to Catalonia (Spain): 2001–2006

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Objective: To describe the epidemiology of malaria imported to Catalonia in six years (2001–2006).

Methods: In this retrospective study, we investigated malaria using data provided by database reports of illness of obligatory declaration from 2001 to 2006. Epidemiological data were analysed by age, sex, country of origin, country and date of trip, reason of travel, chemoprophylaxis, type of malaria, specie of plasmodium, treatment, length of hospital stay and outcome.

Results: During the study period, 1000 cases were notified in Catalonia, 94% (944) laboratory confirmed. Missing information or indeterminate results affected all variables. Almost all malaria cases (99.8%, 936) were imported: 87.6% (784) from Africa, 8.7% (78) from Central and South America, 3.6% (32) from Asia and 0.1% (1) from Oceania, by 24% (192) Spanish citizens and 76% (623) immigrants. Forty-nine percent of cases (441) were imported from Western Africa. In 80% (674) of patients *P. falciparum* aetiology, 14% (120) *P. vivax*, 4% (32) *P. ovale* and 2% (15) *P. malariae* were confirmed. Among the subjects, 60% (600) were men. The youngest patient was 5 months and the oldest 92 years old, 52% of patients (522) were in the age group 21 to 40 years old. Most common reasons for travel were individual travel 43% (254), tourism 37% (219) and professional or missionary travel 17% (102). In 25% (210) cases some kind of chemoprophylaxis was documented, but only 29% (60) specified which drugs were taken and only 10% (21) of cases did it correctly. Chemoprophylaxis consisted in mefloquine in 57% (85) of cases, chloroquine phosphate in 12% (18) and doxicicline in 7% (10). Fifty-nine percent (533) of patients required hospitalisation and 16% (75) of them stayed at the hospital more than 7 days. Four cases of malaria death were reported, all of them due to *P. falciparum* infection. These patients were Spanish adults that travelled to African countries and did not take chemoprophylaxis. Two hundred three children living in Catalonia and travelling to their parents' endemic country of origin became infected by *Plasmodium* spp., 55% (111) of them did not take antimalarial drugs.

Conclusions: During the period 2001–2006 in Catalonia *P. falciparum* was the main aetiology. Western Africa is the region and the continent from which most cases are imported. Immigrants contribute with most of cases. Children and Spanish adults deserve special attention because they often do not take preventive measures.

P747 Pyrosequencing for *Plasmodium falciparum* resistance in returning travellers, refugees and immigrants to Alberta, Canada

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Objectives: Travelers to malaria endemic areas, refugees and immigrants infected with malaria can provide information on the resistance patterns of circulating parasite strains. In 2007 we received 13 blood samples from 8 patients with *Plasmodium falciparum* malaria. These samples were tested for malaria and speciated by real-time PCR. Samples that were positive for *P. falciparum* were genotyped at the pfdhfr locus by pyrosequencing. In this study, we have examined three single nucleotide polymorphisms (N51I, C59R, and S108N/T) associated with resistance to the antimalarial sulfadoxine-pyrimethamine (SP).

Methods: DNA was extracted from 40 microlitres of whole blood using the PSS Bio Instruments 12GC or the Qiagen QIAamp Blood Mini Kit. Real-time PCR was carried out as described (Rougemont et al., 2004) with some modifications. Primers used for pyrosequencing were modified from those designed by Zhou et al. (2006).

Results: All of the malaria strains tested showed at least one mutation in the pfdhfr locus; the majority of the strains showed mutations in all three codons. One traveler to Papua New Guinea had been infected with parasites carrying two mutations in the pfdhfr locus: 59R, and 108N, consistent with high levels of resistance to SP in this area. Sibling refugees from Sierra Leone harbored parasites with the triple mutations 51I, 59R, and 108N. Both developed symptomatic malaria upon arrival in Canada. These genotypes correlate with high SP failure rates documented for Sierra Leone (Checci et al., 2005).

Conclusion: Molecular surveillance of *P. falciparum* in returning travelers, refugees and immigrants can contribute valuable genotyping data to global sentinel networks on the prevalence of drug resistant strains in those regions. Future work will include developing pyrosequencing protocols for other mutations associated with drug resistance, including pfATPase6 and pfcytb.

P748 Chloroquine susceptibility phenotype and virulence of *Plasmodium falciparum*

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Objective: In order to study the relation between *Plasmodium falciparum*'s virulence and phenotype, Chloroquine® resistant or susceptible, we postulated, based on the Fitness concept, that the susceptible phenotype would be more virulent in-vivo than the resistant one and thus more predictive of severe malarial episodes.

Methods: A case-control study on severe malaria imported into metropolitan France over the period of January 1st 1996 and September 30th 2006. Cases and controls were defined as subjects manifesting severe and non severe malarial episodes respectively.

The world health organisation classification, 2000, was used to assess the severity of malarial episodes. The criterion of >4% of peripheral parasitism in non-immune patients was excluded among the above criteria. All patients fulfilling this condition, solely, were regarded as manifesting non severe malaria. Using a bilateral 80% statistically powered test to detect a minimum odds ratio (OR) of 2.0, the number of subjects required was estimated to be 91 severe and 364 non severe cases. Independent risk factors of severe malaria, and subsequently virulence assessment of *Plasmodium falciparum*'s phenotypes, were determined using multiple logistic regression models.

Results: Our study population consisted of 81 severe and 840 non severe cases. The threshold of 80% statistical power required for an appropriate analysis was respected by recruiting 921 subjects thus increasing the case-control ratio to ~1/10. Regarding severe falciparum malaria, the Chloroquine® susceptible phenotype turned to be a protective factor compared to the Chloroquine® resistant phenotype. After controlling for age, level of parasitism, continent of infection and native zone, the resistant phenotype was significantly associated with an increased risk of severe malaria with an OR of 1.85 (95% CI: 1.09–3.1). This result was controlled and consistently confirmed in several logistic regression models including and excluding missing data.

Conclusion: Virulence and Fitness do not seem to correlate. The adaptive advantage of the Chloroquine® susceptible phenotype, according to the Fitness concept, does not result in more enhanced in-vivo virulence. This is the first study to address this subject without any restrictions of age or of aetiology of severe malaria.

P749 High proportion of microscopically confirmed highlands malaria in Eldoret, Kenya (2450 metres)

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Introduction: Malaria is a serious public health problem with social and economic consequences in sub-Saharan Africa in altitude under 1500–2000 m. Despite of altitude higher than 2000 m, cases of non-imported malaria were observed sporadically in 1990 as "highlands malaria". The reason why malaria is ascending to higher altitudes is global warming, agricultural and hydrological changes in combination with other causes.

Methods: We investigated the proportion of confirmed malaria cases among suspected non-imported malaria cases in a community health

centre in Eldoret, Kenya. Within 12 months (6/2005–5/2006) 16484 patients in OPD were seen.
Results: In 4985 patients (30%) malaria was clinically suspected and microscopic investigation of blood smear with direct microscopy and Giemsa staining of peripheral blood was performed. Of 4985 suspected cases, 1939 (39%) were positive. All patients received ACT (artemisine combination therapy) – CoArtem (Artesunate/Lumefantrine), no death on malaria was observed and loss of follow up was common. Also no single case of cerebral malaria was noticed. Cure rates were up to 99% when practically all patients responded to initially therapy.
Conclusion: "Highlands malaria" is a daily reality and up to 2000 (12%) cases a year among more than 16000 patients visits is seen even in altitude about 2500 m.

P750 Usefulness of polymerase chain reaction in the diagnosis of asymptomatic *Plasmodium* infection

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Background: In malaria-endemic countries with stable transmission of malaria, semi-immunity is usually acquired after childhood. Infected adults have either uncomplicated malaria or asymptomatic parasitaemia. This fact is frequent in immigrant population
Methods: During the period of one year we screened 117 immigrants to investigate the presence of asymptomatic malaria. In all patients we realised microscopic examination of thin and thick blood smears, determination of plasmodium antigen (BINAX NOW®) and nested-PCR.
Results: During the period of the study we diagnosed 5 cases of asymptomatic malaria in five patients (4 women, one man). The other techniques were negatives. The countries of precedence were: Equatorial Guinea (two cases), Senegal, Brazil and Ivory Coast (one case respectively). The mean of stay in Spain was 852 days (limits 123–1825). No patients come back to native countries in this time. In three cases the nested-PCR was positive to *Plasmodium falciparum* and in two cases *Plasmodium malariae*. No patients showed symptoms or analytic disturbance. All patients were treated with quinine and doxycicline. No patients dead. After the treatment the PCR became negative in all patients.
Conclusions: Asymptomatic *Plasmodium* spp. parasitaemia in immigrants proceedings of malaria-endemic country is frequent. PCR appears to be a useful method for detecting asymptomatic malaria or low parasitaemias in immigrant patients. This fact is very important in detection and management of imported malaria.

P751 Can non-specific biochemical tests improve the diagnosis and contribute to prognosis in imported malaria?

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Objectives: The objective of this study was to evaluate role of nonspecific biochemical tests in malaria diagnosis, according to their sensitivity, specificity and positive predictive values. In addition, comparison with parasitaemia, occurence of complications, recovery duration and organ failiure was examined to determine possible prognostic value of routine biochemical parameters.
Methods: A retrospective study included 64 patients, diagnosed and treated for malaria in Institute for Infectious and tropical diseases KCS, from January 1999. to December 2006. and healthy control group (n = 11).
Results: Thrombocytopenia, leukopenia, hyperglycaemia, hypocholesterolaemia and elevation of transaminases, lactate dehydrogenase (LDH) and creatinine were associated with malaria. Although thrombocytopenia had highest sensitivity (84%), hyperglycaemia, leukopenia and hyperbilirubinaemia were the most specific (100%) and with the strongest positive predicitive values (100%). Malaria complications were associated with anaemia, leukopenia, thrombocytopenia, hypoproteinaemia, hyponatraemia and elevation of LDH and creatinine. Hypocholesterolaemia, anaemia and fibrinogen elevation had a significant effect on recovery duration. Hepatosplenomegaly in malaria was associated with thrombocytopenia and LDH elevation, as jaundice was associated with anaemia.
Conclusion: Nonspecific laboratory test are useful for prompt and accessible orientation and avoidance of misdiagnosis in febrille travellers returning from endemic regions. Furthermore they proved as valuable auxiliary diagnostic and prognostic tools in combination with the microscopic detection of parasites.

P752 Estimating parasitaemia of *Plasmodium falciparum*: UKNEQAS experience

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Objectives: Approximately 10 percent of the malaria cases from the United Kingdom are seen at the Hospital for Tropical Diseases. The remainder of cases are widely distributed throughout the UK, thus any haematologist can be called upon to diagnose malaria in their laboratory. UKNEQAS Blood Parasitology Scheme was established in 1986, part of its remit was to assess the accuracy of the estimation of malaria parasitaemia the UK. Participants in this EQA scheme are required to identify blood parasites present in either thick or thin films and to estimate the percentage parasitaemia of *Plasmodium falciparum* if appropriate.
Methods: Eight distributions each containing one Geimsa stained thin blood film and/or a Field's stained thick film are dispatched annually to 300 laboratories in both UK and overseas to examine for blood parasites. Participants are expected to estimate the percentage parasitaemia, in the blood films containing trophozoites of *Plasmodium falciparum*, by expressing the number of infected cells as a percentage of the red blood cells.
Results: Results of participants' performance for the diagnosing and estimation of the percentage parasitaemia of *Plasmodium falciparum* were analysed over a fifteen year period. It was noted that between 16 and 58 percent of participants failed to estimate the percentage parasitaemia, between 7 and 28 percent overestimate it, between 2 and 10 percent underestimate and between 1 and 7 percent misidentified the malaria species present. It was also noted that the higher the parasitaemia, the more likely it was to be overestimated.
Conclusion: Although the analyses of results achieved by participants for other blood parasites has shown an overall improvement, estimation of the percentage parasitaemia of *Plasmodium falciparum* remains unchanged. One reason for this is the failure of many participants to specify the percentage parasitaemia. Of those that overestimate the parasitaemia, it is assumed to be due to counting the number of trophozoites per 100 red blood cells and not the number of parasitised red blood cells.

P753 Polymorphism of merozoite surface protein-3α gene of *Plasmodium vivax* in isolates in Iran

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Introduction: The world wide distribution of *P. vivax* has expanded significantly and the number of cases reported has been on the rise. Approximately 88% of malaria cases in Iran are caused by *Plasmodium vivax*, and in order to management of the disease, understanding the population genetic structure of the parasite is necessary for designing and applying drugs and vaccines. Among many potential candidates, merozoite surface protein-3α gene (PvMSP-3α) is promising target to develop an effective vaccine.
Objectives: This study was designed and carried out to determine the variation of this gene, as a genetic marker, in *Plasmodium vivax* isolates in malarious areas of Iran.
Method: Diversity in PvMSP-3α gene was assessed in 85 *Plasmodium vivax* isolated from four southern and east-southern provinces of the country by PCR/RFLP method. Amplification was performed with two primer pair sets in a nested PCR format and the products were digested by the Enzyme HhaI in RFLP method.

Results: Based on the size of the PCR products, we observed 3 biotypes A (about 1900bp), B (about 1400bp) and C (about 1100bp) of PvMSP-3α gene. Biotype A was predominant. According to RFLP patterns, 10 allelic groups of the gene were observed, that, 7, 2 and 1 groups correspond to the biotype A, B and C, respectively. Mixed genotype and multiple infections were not seen.
Conclusion: We found that RFLP method with HhaI enzyme is a useful method for determining the polymorphism of biotype A of PvMSP-3α gene.

P754 Risk factors and epidemiology of malaria in endemic zones of Venezuela

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Malaria is the infectious disease with the highest prevalence in the world. In Venezuela, malaria is produced by *Plasmodium falciparum*, *P. vivax* and rarely *P. malariae*. Here, we carry out a metanalysis with all the available data from six different studies from our research group in different areas of Venezuela, as well as all the available information from published and unpublished sources, in order to characterise the risk factors and the epidemiological trends of the malaria disease. The diagnosis of malaria is commonly performed by the microscopic examination through a network of diagnostic centres. OptiMAL, Parasight and ICT have also been used in the field and for comparison reasons with sensibility and specificity values above 85 and 95%, respectively. PCR has also been used for population based and comparison studies, showing a great diagnostic power. In the endemic areas, all these techniques have detected asymptomatic infections caused mainly by *P. vivax*, and PCR have detected a higher proportion of mixed infection, which are most likely playing an important role in the permanence of disease. Malaria incidence in Sucre state was very high between the years 2000–2002, but after 2003 has declined progressively to be close to eradication in most areas. The main transmission factor here is the geographic condition with large swamp areas and the poverty of the communities. In Bolivar, the incidence have always been high, but since 2004, it has become an epidemic grown out of proportions, producing over 90% of the cases for Venezuela, and irradiating to other states. Here, as well as in Amazonas, the main transmission factor is the large selvatic area, the poorly developed health infrastructure and the migration pattern shown by the natives and illegal miners. The vector commonly reported here is A. darlingi, but recently, new species have been pointed out as vectors. Even though Venezuela has been an endemic area for a long time, our results have shown that a large proportion of people do not know how to prevent the disease. The analyses of age group, sex, occupation and cultural habits as risk factors have shown different patterns depending on the studied areas. The use of impregnated mosquito nets, shown to be a very successful prevention measure in many countries, is not widely used in the endemic areas. The official control measures are usually limited to insecticide spaying and early diagnosis.

P755 Malaria in Tunisia: epidemiology, clinical aspects and management

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Objectives: To study the epidemiological, clinical, laboratory features and management of malaria in Tunisia where the disease has been eradicated since 1980. All the cases were imported, mainly from African countries.
Methods: Medical records were reviewed for patients with microscopically confirmed malaria diagnosed at our department during a twenty years period (from august 1986 to december 2006).
Results: 110 patients were included in the study. 90.9% of them were male, and median age was 30 years (range, 14–57). 52.7% of patients were caucasian.34.5% of them had history of malaria and were from Central Africa. Only 26% of cases had taken chemoprophylaxis with poor compliance, mainly in tunisian travellers. Of a total of 110 cases, 87 were caused by *P. falciparum*, 14 by *P. vivax*, 3 by *P. ovale*, 2 by *P. malariae*, 2 patients had dual infection and in 2 cases plasmodia were unspecified. 97% of cases were acquired in subsaharian Africa. All immigrants living in Tunisia have acquired infection in their countries of origin and develop symptoms after a journey. Tunisian people were infected outside the country while travelling for many reasons. 94% of patients gave history of fever; 29% however were afebrile at the time of their presentation. Other commonly but unspecific symptoms were headache, chills, myalgia and gastro-intestinal troubles. 38% of cases had jaundice.
Thrombocytopenia (<150,000/mm3) was the most prominent laboratory feature occurring in 41% of cases. Hemoglobin levels below 12 g/dl were observed in 22%. Leucopenia (<4000/mm3) was noted in 22%. Leukocytosis in 7%. ALT (>22U/l) was elevated in 40% of cases.
Diagnosis of malaria was made in asymptomatic African students in 7 cases. 2 patients (Tunisian) had severe falciparum malaria requiring treatment at the ICU.
For *P. falciparum* cases, mefloquine was the drug of choice, whereas chloroquine associated to primaquine was used for other species. All the patients recoverd well.
Conclusion: Malaria remains a major public health problem in many subsaharian countries. Due to the increased number of international travellers for many reasons, we should be altert to the reintroduction of the prevent disease in our country.

P756 Malaria: between two ports

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Introduction: For our hospital geographic localisation, between port of sea and airport, ill travelling people are referring to us.
Objectives: To evaluate clinical epidemiology aspects and evolution of the malaria cases.
Methods: Retrospective revision of the interned malaria cases in the last 6 years (January 2001 to April 2007).
Results: In this period 18 malaria cases had been interned (15 men: 3 women), 6 had occurred in 2006; average ages 43±14 (16–62) years. All with recent stay in Africa (Angola, Mozambique, Cameroon, Coast Ivory and Libyan), without previous prophylaxis; 10 live there, 4 had travelled and 4 were sailors embarked that had arrived in port there. In 72% the responsible parasite was *P. falciparum*, 11% *P. vivax* and in 17% was not possible identification. The most common manifestations had been gastrointestinal symptoms, fever and chills. They had presented thrombocytopenia (100%) and anaemia (78%). The complications had been acute renal insufficiency, cerebral malaria and ARDS. That had occurred in no resident endemic area people, with high parasitaemia (10, 20 and 35%) and in a pregnant woman. Patients with complications had necessity of intensive care. They had been treated with quinine and doxycycline, plus primaquine in the others species. The bed average was 8±5 (4–29) days. Mortality was 5.5% (1 case), with favourable evolution in the others.
Conclusions: In Portugal, malaria is an imported disease, most origin in Africans countries of Portuguese official language, and in our case 22% in embarked sailors. As related in literature, the severe cases had happened in not residents, high parasitaemia and predispose conditions. Prophylaxis measures must be taken, especially in travellers and sailors who arrived to endemics areas.

Immunology, host defences and immunotherapy

P757 Differential expression of Toll-like receptors in chronic hyperplastic candidosis

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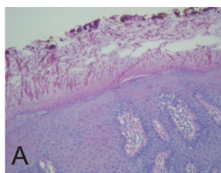
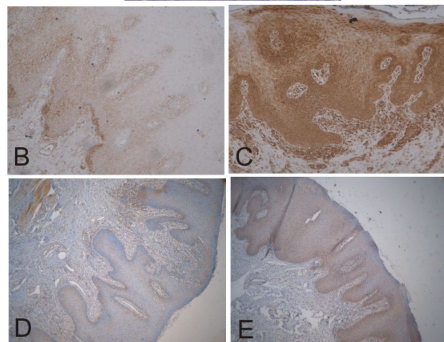
Introduction: The first step in the host defence against oral candidosis is recognising *Candida albicans* through a set of germ-encoded pathogen recognition receptors e.g. Toll-like receptors (TLRs). In man, ten types of such receptors have been identified so far out of which only TLR2, TLR4 and TLR6 have been linked to mediating Candidal ligands e.g. zymosan and mannan.
Materials and Methods: Biopsies from patients with chronic hyperplastic candidosis (n = 5), leukoplakia (n = 5) and healthy mucosa (n = 5) were immunohistochemically stained with the full panel of TLRs (TLR1–9) antibodies to visualize and compare the staining pattern of the epithelial layer in the three categories of tissues.
Results: On analysis, the epithelium of all tissues was divided into three layers: basal, middle and su-perficial. Two of the five chronic hyperplastic candidosis sections showed high numbers of hyphae compared to yeasts, which paralleled a decrease in the expression of TLR2 and an increase in the stai-ning intensity of TLR4. Leukoplakia and healthy tissue sections demonstrated stronger immunostaining of TLRs except TLR9 for the first and the basal layers for the latter.





Discussion: This study supports the concept of negative regulation of TLRs which, for long time, are either ligand-bound (e.g. in chronic hyperplastic candidosis), or not stimulated (in healthy tissue). It also augments the opinion which says that *Candida albicans*, through its hyphae rather than conidia, may exploit TLRs i.e. TLR2, to evade the immune system of the host. Leukoplakia seems to be more alert to lessen the chances of worsening the already-diseased tissue.

P758 Polymorphisms in the mannose-binding lectin and in the MBL-associated serine protease genes in intensive care unit patients with systemic inflammatory response syndrome

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Background: Polymorphisms in the mannose-binding lectin (MBL2) and in the MBL-associated serine protease (MASP2) genes have been associated with an increased risk of infections. The aim of the study is to asses the possible association between polymorphisms in these genes and the frequency of infections and death among intensive care unit (ICU) patients with systemic inflammatory response syndrome (SIRS).
Methods: Blood samples from 243 consecutive UCI patients with SIRS (mean age 62.75±15.69 years; 63% men) admitted in our hospital from January 2003 to January 2004 were prospectively collected. For further comparison samples from 104 blood donors were also included in the study. At entry, 94 patients exhibited septic shock, 29 severe sepsis, 49 had sepsis and 71 had a noninfectious SIRS. Mean APACHE II, SAPS II and SOFA score at ICU entry were 17.4 + 6.3, 36.5 + 11.8, 8.7 + 3.1, respectively. In-hospital rate mortality was 30%. Six single nucleotide polymorphism's (–550 G/C, –221 C/G, +4 C/T, codon 52 CGT/TGT, codon 54 GGC/GAC and codon 57 GGA/GAA) in the promoter and the exon 1 of the MBL2 gene and the Asp 105 Gly single nucleotide polymorphism's in the MASP2 gene were genotyped using a sequence-based typing technique.
Results: No significant differences were observed in the frequencies for low expression MBL2 genotypes and for the wild type MASP2 genotype between patients with SIRS (13.02% and 94.4%, respectively) and the healthy controls (15.3% and 97.1%, respectively) (P= 0.86 and P= 0.6, respectively; Chi square test). Although patients with an infectious cause of SIRS had a higher incidence for low expression MBL2 genotypes (15.7%) compared to those with a non infectious SIRS (6.5%), these differences were not statistically significant (P= 0.064; Chi^2 test). No differences were found regarding the frequency for low MBL2 among patients with septic shock or with severe sepsis or among non-survivor ICU patients. The presence of polymorphism's in the MASP2 gene was not associated with a higher rate of infections or mortality.
Conclusions: Although patients with MBL2 genetic polymorphisms causing low MBL2 levels have a tendency to present a higher incidence of an infectious cause of SIRS, the presence of MBL2 or MASP2 genetic polymorphism's do not seem to have a significant impact neither in mortality nor in the severity of infections in ICU patients with SIRS.

P759 Toxin production by meticillin-resistant *Staphylococcus aureus* strains is related to differences in cytokine levels by peripheral blood mononuclear cells

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Objectives: To investigate the immune system response in terms of secreted cytokines, to the existence of staphylococcal virulence factors among meticillin-resistant *Staphylococcus aureus* (MRSA) isolates.
Methods: Twenty-two, 18 clinical and four reference MRSA strains were characterised on the basis of toxin gene profile by PCRs (PVL, tst, enterotoxins of the egc operon, eta, etb). Clones were defined by agr typing and MLST, while tst expression was calculated by quantitative reverse-transcription PCR (RT-PCR). Peripheral blood mononuclear cells from healthy donors were isolated by standard procedures and were co-cultured in 24-well flat bottom tissue culture plates with live MRSA cells at a ratio of 25:1. Cytokine measurements, using the Luminex® xMAP™ technology, were performed in cell-free supernatants collected at 6 and 12 hours. The 8-plex assay kit was used for simultaneous quantitative determination of the following cytokines: TNFa, IL-1b, IL-6, IL-8, IL-10, IL-12p40, IL-13 and GM-CSF.
Results: MRSA elicited production of detectable amounts of all eight cytokines. A negative correlation was detected between PVL genes carriage and production of GM-CSF at six hours, and TNFa, IL-6, IL-10, IL-13 and GM-CSF at 12h. The presence of tst gene was correlated with release of greater amounts of TNFa, IL-6, IL-8, IL-10, IL-13 and GM-CSF at 6 and 12h. The presence of tst gene correlated with lower levels of IL-12p40 at 12h. Expression level of tst and presence of the remaining superantigens were not related to cytokines' levels. Analysis of agr types and cytokine profile showed that MRSA of agr1 (including tst-positives) induced production of greater amounts of TNFa, IL-6, IL-8, IL-10, IL-13, GM-CSF and IL-12p40 at 12h than agr2 (including egc-positives) and agr3 (including PVL and egc-positives) MRSA. All differences were statistically significant ($p < 0.05$).
Conclusions: Our data reinforce the knowledge that toxic shock syndrome toxin-1 has strong proinflammatory and immunoregulatory properties, perhaps in association with other factors, as it directs immune

system to TH2 response by promoting the release of IL-10 and IL-13 and down-regulating the production of IL-12p40. PVL has a diminished potential towards triggering immune responses, as strains possessing PVL genes elicited production of lower amounts of pro-inflammatory cytokines and cytokines of adaptive immune response.

P760 Influence of combined contraceptive vaginal ring on vaginal microflora and immunity

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Objectives: to establish influence of vaginal ring on microbiocenosis of low female reproductive tract.
Methods: 31 female women age of 20–25 years old were examined before and after 3-month usage of contraceptive vaginal ring (NuvaRing). Vaginal ring is combined low-dosed hormonal contraceptive which releases 120 μg of etonogestrel and 20 μg of ethinyloestradiol daily. The samples of vaginal discharge were inoculated into a culture medium (Columbia base agar, blood agar, Man Rogosa Sharpe medium, semisynthetic growth medium, McConkey agar, *Gardnerella vaginalis* agar and Sabouraud agar) and cultured for anaerobic and aerobic bacteria. Secretory immunoglobulin, IFN-gamma, IL-4, lysozyme in cervico-vaginal mucus were detected by ELISA. Data were analysed using Statistica 6.0 (StatSoft, USA).
Results: After vaginal ring usage considerable changes of vaginal microbiocenosis structure were observed. Amount of *Lactobacillus* increased from 10^4 to 10^6 colony forming units (CFU) and *Bifidobacterium* from 18.2±1.2 to 44.4±6.1 CFU ($p \leqslant 0.05$). Percentage of women, having three-component microbial association (*Peptostreptococcus*, *Staphylococcus* and *Streptococcus*) decreased from 66.7±8.8% to 46.7±9.1%. Nevertheless, number of *Candida albicans* increased from 10^2 to 10^4 which gave rise to symptomatic yeast infection.
Elimination of cocci during NuvaRing usage could be consequence of elevation of secretory immunoglobulin (from 47±12.5ng/ml to 94.5±22.1 ng/ml) and IL-beta (from 95.7±29.1 pg/ml to 130.2±29.1pg/ml) ($p \leqslant 0.05$). High rate of *Candida* detection, apparently, could be result of high local level of estrogen among women using contraceptive vaginal ring.
Conclusion: Thus, our studies showed increasing concentration of lactobacilli and bifidobacterii, and cocci elimination, wich is favourable for antiinfectious resistance of female reproductive tract. Meanwhile, high risk of yeast infection appearance doesn't make possible to recommend this type of contraception for women with recurrent candidiasis, diabetus mellitus, AIDS and etc.

P761 Immunmodulatory potencies of linezolid in a LPS model of human whole blood

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Objectives: Gram-negative sepsis resulting in septic shock is one of leading causes of death in critically ill patients. One important determinant of mortality is the release of endotoxin, which is a structural component of LPS. It is generally accepted, that the first mediators in the cascade of LPS-induced events are cytokines, like TNF-alpha and IL-1beta. Because treatment options are limited, a wide range of therapeutic strategies are still under investigation, but convincing reduction of mortality is still lacking. New approaches are focused on immunmodulatory effects of antimicrobials. Several studies investigating Linezolid in different models of Gram-negative sepsis showed significant reduction of pro-inflammatory cytokines but used un-physiologically high doses of LPS. The aim of our study was to characterise the immunmodulatory effects in a model of human whole blood with concentrations of LPS and Linezolid reflecting physiological conditions.
Methods: Whole blood from 10 healthy volunteers was incubated with 50 pg/mL LPS W/o 13 μg/mL Linezolid for 2 and 4 h. RT-PCR was performed from messengerRNA (mRNA) of IL-1β, IL-6 or TNFα, 18sRNA was used as control. Protein levels of the supernatant were measured using ELISA for IL-6 and TNFα.
Results: Incubation of human whole blood with LPS significantly increased mRNA levels of cytokines compared with baseline. The addition of Linezolid significantly reduced mRNA levels of IL-1β (−49%; $p < 0.01$), IL-6 (−49%; $p < 0.01$) and TNF-α (−61%; $p < 0.02$) after 2 hours. In addition, after 4 hours reduction on mRNA levels of IL-1β (−49%; $p < 0.01$), IL-6 (−25%; $p < 0.03$) and TNF-α (−32%; $p < 0.04$) were observed. Although LPS stimulation increased levels of IL-6 and TNFα between 100 and 1000-fold, in contrast to mRNA no reduction by addition of Linezolid was observed on protein level.
Conclusion: The previously assumed immunmodulatory effects of Linezolid were confirmed in our in vitro sepsis model on gene expression level. However, differences to previous findings which have shown significant reduction on protein levels were observed. This discrepancy might be mainly ascribed to methodological differences, since compared to previous work more physiologic conditions were employed in the present setting.

P762 Influence of linezolid on phagocytosis and killing of Gram-negative bacteria by polymorphonuclear neutrophils

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Objectives: In a recent phase III clinical trial on linezolid it became obvious that in the linezolid treatment arm more patients acquired Gram-negative catheter related blood stream infections. It was postulated, that linezolid may trigger the acquisition of Gram-negative infections associated with higher mortality rates despite adequate anti-Gram-negative therapy. Therefore, we analysed the impact of linezolid on phagocytosis and killing of Gram-negative bacteria by polymorphonuclear neutrophils (PMN). Phagocytosis and killing of pathogenic microorganisms by PMN are of high relevance for the prognosis of patients suffering from bacterial infections.
Methods: Whole-blood samples were taken from healthy volunteers and incubated with linezolid in clinical relevant concentrations (5, 20 and 50 mg/L) and compared to a drug-free control. CD11b-expression of PMN was evaluated using fluorescence-labelled mAB. To measure phagocytosis, fluorescence-labelled Gram-negative bacteria (*Escherichia coli*, *Burkholderia cepacia*, *Pseudomonas aeruginosa*) and Gram-positive control (*Staphylococcus aureus*) were added to the blood. Bacteria : PMN ratio was adjusted to 5:1. Phagocytosis was stopped after 5, 15, 30, and 60 min, respectively. Samples were subsequently analysed by flow-cytometry.
To evaluate the influence of linezolid on killing of bacteria whole blood was incubated with 0 (control), 5, 20 and 50 mg/L linezolid, respectively. Killing of bacteria in whole blood was determined after three hours of incubation by plate counting.
Results: Linezolid impaired CD11b-expression of PMN in a concentration-dependent manner up to about 15 percent. Additionally, linezolid also impaired phagocytosis of *E. coli* (5 to 29%) and to a lower extend of *B. cepacia* (⩽ 15%) and *P. aeruginosa* (⩽ 10%) in a concentration-dependent manner. In contrast killing of tested Gram-negative and Gram-positive bacteria were not affected by linezolid compared to the control.
Conclusion: Our data reveal a negative, concentration-depended influence of linezolid on the CD11b-expression of PMN and on phagocytosis of *E. coli* and to a lower extent of *B. cepacia* and *P. aeruginosa* but not of *S. aureus*. Despite no observable influence of linezolid on the killing of *S. aureus*, *E. coli*, *B. cepacia*, and *P. aeruginosa* an impaired phagocytosis of Gram-negative bacteria might be critical, especially, in patients with otherwise impaired immune system even if patients receive additional adequate anti-Gram-negative therapy.

P763 Granzyme K levels and NK cell lytic activity in experimental mice challenged with LPS and/or IL-2+IL-12

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Objectives: Widespread activation of the innate immune system by infecting pathogens results in uncontrolled systemic release of an array

of inflammatory mediators that generate clinical sepsis. Recently, we found that the levels of Granzyme K (GrK), a potent lymphocyte serine protease, correlate with the stage of sepsis but, the reason for increased GrK levels during inflammatory responses/sepsis is unclear. Since natural killer (NK) cells, which are know to be critically involved in the course of sepsis may also be responsible for the production of increased GrK levels, the aim of the present study was to investigate the changes in GrK levels along with the NK cells lytic activity in experimental animal models.

Methods: Recipients C57BL/6 mice were injected with bacterial lipopolysaccharide (LPS, 25 μg) in the first experimental model. In the second model, inflammatory response was induced in recipients by combined injection of cytokines, interleukin (IL)-2 and IL-12 (3×10^5 U rhuIL-2 + 1 μg rmuIL-12) which were also shown to be synergistic inducers of granzymes message. Our previously developed GrK-ELISA system was used to measure the plasma levels of GrK in LPS and/or IL-2+IL-12 injected recipients. Subsequently, the NK cells lytic activity was examined by testing the ability of single cell suspension prepared from recipient spleen cells to lyse the NK sensitive target, 51Cr-labeled YAC cells.

Results: Increase in GrK levels in mice plasma follow the increase in NK cell lytic activity in LPS challenged mice while in IL-2+IL-12 challenged mice no significant increase in NK cell lytic activity was observed. Furthermore, the changes in GrK levels in mice plasma of both experimental models were found to correlate with the changes in GrK levels of septic patients.

Conclusions: GrK could constitute at least one of the mechanisms by which NK cells contribute to systemic inflammation/sepsis. In addition, the results confirmed the role of GrK in sepsis pathology and further indicate that it may be a potential diagnostic marker and/or novel therapeutic target for further treatments of this life-threatening disease.

P764 Chronic infection with hepatitis B virus is not associated with early senescence of CD8+ T lymphocytes

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Objective: To assess whether CD8+ T lymphocyte phenotype in patients with chronic hepatitis B virus (HBV) infection is compatible with accelerated senescence, as observed in infection with other persistent viruses, namely cytomegalovirus (CMV).

Methods: T lymphocyte subpopulations have been quantified in separated peripheral blood mononuclear cells from 24 chronic hepatitis B (CHB) patients (age 18–56 years), 28 healthy age-matched blood donors and 16 convalescent patients with acute hepatitis B (AHB). Expression of CD45RA and co-stimulatory molecules CD27 and CD28, determined by 4-colour direct immunofluorescence and flow cytometry, was used to establish the maturation phases of CD8+ T lymphocytes according to the sequential loss of CD45RA, CD28 and CD27 (combined with re-expression of CD45RA during quiescence) according to the model proposed by Appay and Rowland-Jones (Semin. Immunol.2004;16:205–212). Percentages of lymphocytes in each subset were compared by Student's t test and the differences scored as significant by $p < 0.01$.

Results: Comparable percentages of CD45RA+ CD8+ T lymphocytes were found in healthy controls (60±15% of CD8+ T cells) and CHB patients (65±14%), but AHB patients showed significantly lower percentages (46±19%). Truly naive (CD45RA+ CD27+ CD28+) CD8+ T lymphocytes were significantly more represented in CHB patients (23±16%) than in both healthy controls (11±11%) and AHB patients (11±7%). The AHB patients showed a relative expansion of the recently activated antigen-experienced (CD45RA- CD28- CD27+, intermediate phenotype) CD8+ T cells whereas the supposedly quiescent analogous subset (CD45RA+ CD28- CD27+) was significantly smaller in the CHB patients (20±12%) compared with healthy controls (36±21%). No significant difference was found in the late (CD28− CD27−) antigen experienced subsets.

Conclusion: The data suggest that chronic infection with HBV is not associated with accumulation of terminally differentiated CD8+ T lymphocytes and a corresponding early senescent phenotype, but otherwise by a larger input or accumulation of naive lymphocytes in peripheral blood.

P765 Generation of influenza recombinant haemagglutinin-specific lymphocytes from cordblood-derived dendritic cells: immunotherapy model from an unprimed source

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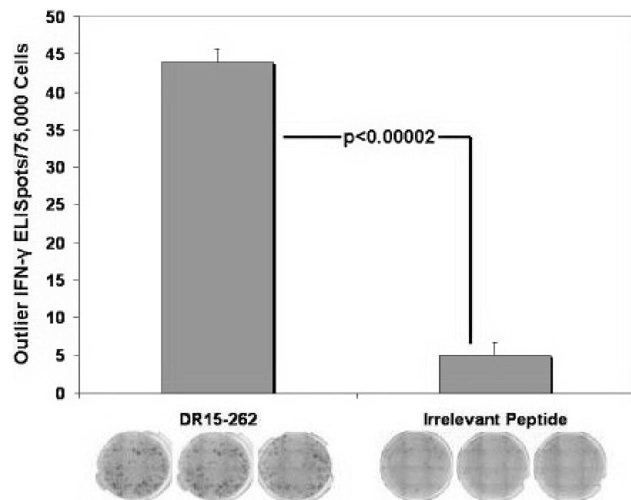
Background: Cordblood-derived (CB) is an important source of mostly unprimed stem cells. We sought to generate CB-derived dendritic cells (DC) immunotherapy against influenzavirus.

Methods: Recombinant hemagglutinin (rHA) was expressed in insect (SF9) cells by recombinant baculovirus. The HA gene of influenza A New Caledonia/20/99 (H1N1) influenza virus was independently cloned into the plasmid baculovirus expression vector pPSC12. Generation of Immature DCs. Umbilical cord blood units discarded from the MDACC CB bank were used after IRB approval. DC Loading and Maturation. After six days of culture in GM-CSF and IL-4, immature dendritic cells were loaded with rHA protein. Immature dendritic cells were suspended at a concentration of 4×10^7 cells/ml, mixed with rHA and incubated for 10 minutes on ice in an electroporation cuvette. T-cell Priming and Re-Stimulation. Upon maturation of loaded DCs, they were incubated at a ratio of 1:10 with autologous non-adherent PBMCs (typically 50% CD3+), plus IL-12. ELISpot Assay. Typically, 10^4 to 10^5 lymphocytes from each culture were mixed at a ratio of 10:1 with thawed dendritic cells, plated in triplicate on anti-IFN-gamma coated ELISpot plates (BD Biosciences) and cultured overnight (12–18 hours). ELISpots for with putative HA peptide epitopes were performed similarly using 1×10^5–2×10^5 lymphocytes incubated overnight in 3.75 μg/ml peptide.



Results: rHA-specific lymphocytes demonstrate identifiable HLA-restriction. As demonstrated by Figure, HA-primed lymphocytes (HLA DRbeta1*1503) from a different CB demonstrated a nine-fold increase in statistically large spots when restimulated with the DR15–262 epitope ($p < 0.00002$). These data suggest that 1 in 1,900 of the HA-specific T-cells were DR15–262 restricted in a highly-specific fashion. Total ELISpot numbers ($p < 0.0002$) and total IFN-gamma release ($p < 0.00004$) between lymphocytes restimulated with DR15–262 and the control peptide were statistically distinct as well. Incubation of peptide DR15–262 in conjunction with HA-primed/DR15- lymphocytes or in conjunction with adenoviral hexon-primed (irrelevant) lymphocytes did not demonstrate significant numbers of IFN-γ spots.

Conclusions: The results demonstrate that, despite the generally naive of CB lymphocytes, influenza HA-specific responses can be generated ex vivo and could be potentially be used to enhance immune reconstitution following allogeneic stem cell transplantation.

P766 **Staphylococcal activation of plasmacytoid dendritic cells requires preformed IgG**

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Objectives: Induction of type I interferon (IFN-I) production has previously been detected in response to infection with intracellular bacteria and upon stimulation of human PBMC with extracellularly located *Staphylococcus aureus* or *Escherichia coli*. In spite of these observations the molecular mechanism triggering IFN-I by extracellular bacteria has so far not been elucidated. The aim of the study was to provide information on the mechanism involved in IFN-I induction by staphylococci.
Methods: Human plasmacytoid dendritic cells (PDC) were enriched from PBMC with BDCA4 microbeads. PDC were resuspended in culture medium w/o human serum and stimulated with staphylococci. IFNa secretion was quantified by ELISA. Anti-staphylococcal immunoglobulin was detected by Western Blot.
Results: Here we demonstrate that staphylococcal activation of human plasmacytoid dendritic cells occurs in a species-specific manner. Interestingly, all donors tested responded to *S. aureus* while only few donors responded to *S. epidermidis* and other coagulase-negative staphylococci. PDC activation was subsequently found to depend on the donor serum. We further show that IFNa levels depend on the concentration of preformed antigen-specific IgG. Moreover, *S. epidermidis*-responsive and non-responsive donors can be distinguished by the presence or absence of anti-*S. epidermidis* Ig.
Conclusion: PDC recognition of extracellular bacteria requires a prior humoral immune response with antigen-specific IgG formation. Since IFN-I primes immune cells for a more efficient response to pathogens our data suggest that the human immune response to *S. aureus* is more efficiently unleashed than that to other staphylococci. Furthermore, patients at risk for developing sepsis may benefit from vaccination against common bacterial pathogens.

Pathogenesis and diagnosis of viral infections

P767 **Mapping of the binding domains of the Newcastle disease virus phosphoprotein in P–L and P–(NP-RNA) complexes**

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Newcastle disease virus (NDV) is an avian pathogen which affects the poultry industry worldwide. Outbreaks of the disease known as Newcastle disease (ND) often result in mass culling of the infected chickens. NDV has also oncolytic activities against human malignancies which lead to tumour apoptosis and cell death. Besides that NDV can serve as a viral vector to express and deliver foreign genes which is useful in vaccination and gene therapy. To identify the mechanisms involved in NDV pathogenesis as well as its anti cancer properties, the understanding of the mechanisms involved in the transcription and replication of the virus is of fundamental importance. One way to characterise such mechanisms is to identify the involvement of the viral proteins and their interacting partners. It has been shown in other paramyxovirues that the phosphoprotein (P) plays a central role in the transcription and the replication of the viral genome. Together with the large (L) protein, it forms and stabilizes the active RNA-dependent RNA polymerase complex. It also mediates the interaction between the L protein subunit of this complex with the viral nucelocapsid (NP-RNA) template comprising a nucleocapsid tightly bound RNA genome. Employing a yeast-two hybrid system the presence of P-L interaction in NDV was investigated. However, due to the low expression level of L protein yeast, no interaction was observed. Therefore, an in vitro co-translation approach was carried out to identify P-L interaction. The result showed that NDV P protein is also involved in the stabilisation of L protein. To identify the presence of NDV P–(NP-RNA) interaction and the involved domain(s), co-immunoprecipitation was performed. Purified NP:-RNA template along with in vitro translated P or its derivatives were separately co-immunoprecipitated using anti-His antibody. The results revealed that the immediate N-terminal of P protein is involved in its interaction with NP-RNA template.
In conclusion, the current study has delineated the key interacting partners of NDV P. This knowledge will be useful for structural and functional exploration of this protein.

P768 **Host cellular responses in Nipah virus infection**

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Objectives: Nipah virus (NiV) is a zoonotic infectious virus that causes severe to fatal central nervous system infection in human during an outbreak in Malaysia in 1998. During the outbreak, NiV also causes disease in pigs. Unlike the infection in human, infected pigs normally present with respiratory distress syndrome and the disease is less severe in comparison to human infection. The present study was undertaken to investigate and understand the possible molecular mechanisms that may contribute to the difference in pathogenicity of NiV infection in the two hosts, human and pigs.
Methods: We examined NiV infection and replication in selected cell culture systems representing the two hosts, human and pigs. The state of dysfunction of the NiV-infected cells was then examined using proteomic approaches.
Results: Infection of four different cell types representing the two host organisms using NiV shows that the virus establishes productive infection in all the cell types examined. There are no differences in the capacity to support NiV replication between fully susceptible porcine stable kidney cells (PS) and human lung fibroblasts cells (MRC-5), respectively. However, there are differences between these cells, and human neuronal cells (SK-N-MC) and monocytes (THP-1), in the ability to support NiV replication and virus release. The SK-N-MC cells are less able to support NiV infection when compared to the PS cells. Subsequent examination of the changes in cellular host response as a result of NiV infection in both human and pig cells identified a total of 15 differentially expressed proteins. Most of these proteins are involved in virus replication or RNA synthesis, and proteins associated with the cellular functions of the mitochondria or the induction of apoptosis. The potential importance of these differentially expressed proteins includes the regulation of NiV replication in the two hosts as well as the manifestation of cellular cytopathic responses to the infection.
Conclusion: The proteomic analysis of NiV-infected cells of human and pigs identified differences in the key cellular pathways and events occurring during NiV infection in the two hosts, respectively, that could contribute to the differences in manifestation and severity of NiV infection.

P769 **Crimean-Congo haemorrhagic fever virus infects peripheral blood mononuclear cells and does not disrupt tight junctions in MDCK-1 cells**

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Objectives: Crimean Congo Haemorrhagic Fever is characterised by severe bleeding with mortality rates averaging 30%. There is no verified treatment or prophylaxis making this a grave disease to contract. The molecular mechanisms underlying pathogenesis are completely unknown, however a few theories have been postulated for other viral haemorrhagic fevers. It could be a direct effect of viral infection on cells that can cause haemorrhage (through disturbing tight junctions) or it could be a host mediated effect. If infection disrupts tight junctions, that could be the cause of blood vessel leakage explaining the observed haemorrhages.
In this study we have analysed the effect of viral infection on tight junctions in different model cell lines. We have also studied the ability of Crimean Congo Haemorrhagic Fever Virus (CCHFV) to infect different human cells.
Methods: In order to study viral effect on tight junctions, different polarised cells were grown on Transwell semi-permeable filters and

transepithelial electrical resistance measured. Visualisation of the tight junction proteins occludin and ZO-1 was performed by indirect immunofluorescence microscopy, to study localisation of these proteins upon infection.
Infectivity of cells was verified by western blotting, PCR and indirect immunofluorescence.
Results: Tight junction integrity was analysed by both TER and IF, and there was no measurable disruption indicating viral infection does not disturb tight junction integrity.
Viral protein and RNA could be found in different cells.
Conclusion: There was no measurable effect on tight junction integrity in highly polarised cells.

P770 Replication of Vaccinia virus on PBMC is restricted by mediators of innate immunity

C. Castilletti, L. Bordi, C. Agrati, E. Lalle, F. Carletti, E. Cimini, M.R. Capobianchi (Rome, IT)

Objectives: No detailed studies have been conducted on interactions of poxviruses with the immune system, relevant to clarify pathogenic mechanisms of poxviruses infection, and to improve poxvirus vaccine vectors and immunomodulatory strategies. The aim of our study was to better understand the cellular targets of Vaccinia Virus (VV) infection and their consequences of infection for cells involved in the generation of antiviral immune responses.
Methods: The ability of VV to productively infect normal PBMC and different cell subpopulations (CD14+, CD4+/CD8+ and CD4−/CD8−) was determined by back titration of infectivity and quantitative detection of viral genome in kinetic studies. In the same experiments the extent of induction of IFN alpha and/or gamma, was measured at both mRNA and protein level.
Neutralising antisera against IFN alpha and gamma were used to establish if the low replication rate of VV in PBMC is due to the activation of the IFN system.
Results: VV replication efficiency in PBMC is generally low, and is restricted to few PBMC subpopulation (namely: CD14+ and CD4−/CD8−), while resting T cells do not support VV replication. Neutralising antibodies to IFN alpha and gamma strongly increase the replication of VV in CD14+ and CD4−/CD8− cells, indicating that these cytokines are induced by VV, and are at least partially responsible for restricted replication in PBCM subpopulations.
Conclusions: Our findings indicate that mediators of innate immunity, such as IFN system, are important factors in the control of orthopoxvirus replication in white blood cells, and may be exploited as therapeutic strategy to counteract severe infections, that can occur as result of zoonotic exposure in humans.

P771 Avian influenza H5N1 virus is able to disregulate interferon response activation in human PBMC

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Objectives: The extraordinary spread of highly pathogenic avian influenza A (HPAI) H5N1 viruses has resulted in devastating outbreaks in domestic poultry and sporadic human infections with a high fatality rate. Unlike most other influenza infections, H5N1 infection causes a systemic disease. The underlying mechanisms for this effect are still unclear. Impaired Interferon (IFN) response may be involved in enhanced pathogenicity of HPAI H5N1 infection. We investigate the ability of both H5N1 and H3N2 viruses to trigger the activation of the IFN system in human PBMC in vitro.
Methods: Human, freshly collected, PBMC from healthy donors were exposed to either HPAI H5N1 or H3N2 to assess the extent of induction of IFN alpha and gamma at both mRNA and protein level. Moreover, we used quantitative Real-time RT-PCR to compare the mRNA levels of IFNAR-1 and of downstream cascade of IFN response genes, such as PKR, MxA, OAS.
Results: The HPAI H5N1 virus was more effective than H3N2 in inducing IFN alpha and gamma, at level of both intracellular mRNA and released protein. On the contrary, mRNA for IFN stimulated genes (ISGs) were less effectively induced in PBMC infected with H5N1 virus as compared to H3N2. Moreover, IFNAR-1 mRNA levels were reduced in PBMC exposed to H5N1 as compared to H3N2 and to unstimulated PBMC.
Conclusion: Our data suggest that the H5N1 virus, although being able to induce IFN alpha and gamma to a greater extent than the less pathogenic H3N2 subtype, is able to attenuate the response to IFN alpha by reducing the extent of IFNAR-1 expression, so that the downstream IFN activated cascade is less efficient as compared to H3N2. As IFN system represents one of the first lines of defence against virus infections, the impairment of this critical host innate immune response may contribute to the high virulence of HPAI H5N1 virus in humans.

P772 Comprehensive analysis of genomic sequences of oncogenic viruses in colorectal cancer

V. Militello, L. Barzon, M. Trevisan, C. Militello, G. Palù (Padua, IT)

Objectives: The aim of this study is to investigate whether there is an association between oncogenic viruses and colorectal preneoplastic lesions and carcinomas.
Methods: This study was conducted in tissue samples from 113 patients diagnosed with colorectal polyp or cancer, including 72 archival colorectal cancers and matched adjacent mucosa and biopsies of colorectal cancers, polyps, and adjacent mucosa, which were longitudinally collected from 41 consecutive patients. Biopsies from 10 subjects, who underwent colonoscopy without detection of benign or malignant lesions, were used as controls. The presence of the herpesviruses (EBV, HCMV) and the polyomaviruses (JCV, BKV, SV40) was investigated by quantitative real-time PCR. Detection and typing of HPV was performed by PCR and sequencing.
Results: EBV-DNA was detected in 30% of cancers and polyps (without significant differences between benign and malignant lesions) and in 14% adjacent mucosa (lesion vs adjacent mucosa, $P < 0.05$); HCMV-DNA was detected in 4% of cancers and in 2% of adjacent mucosa (P=NS). In control biopsies, EBV and HCMV DNA was detected in 50% and 10% of cases, respectively. Both EBV and HCMV were detected in tissue samples at very low viral genome equivalents/cell, consistent with lymphocyte/macrophage infiltration. HPV-16 was detected in 4% of cancers (all rectal carcinomas), but not in adjacent mucosa nor in control samples. No polyomavirus DNA was detected in any sample.
Conclusions: This study seems to exclude a role of EBV, HCMV, and polyomaviruses in the pathogenesis of colorectal cancer, whereas high risk HPV-16 could be involved in some cases of rectal carcinoma. These results are at variance with some reports in the literature, which demonstrated a high prevalence of JCV and HCMV sequences in colorectal cancer and suggested these viruses could have a role in tumorigenesis.

P773 Human cytomegalovirus infects adrenocortical cells and stimulates adrenal steroidogenesis

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Objectives: Based on our observation that human cytomegalovirus (HCMV) genome and expression of both early and late genes are frequently detected in functioning adrenocortical tumours, aim of this study was to investigate whether HCMV infects and replicates in adrenocortical cells in vitro and the effects of viral infection on steroidogenesis and adrenocortical cell growth.
Methods: human adrenocortical carcinoma (ACC) cell lines NCI-H295 and SW-13 and primary ACC cell cultures were infected with clinical isolates of HCMV and with the HCMV strain AD169 and analysed by immunofluorescence assay. At different time points, steroid hormones were measured in culture medium, while cells were harvested for RNA extraction and evaluation of gene expression by microarray analysis.

Infected cells were tested with BrdU assay and stained with propidium iodide and annexin-V antibody for the evaluation of apoptosis, cell proliferation, and cell cycle.
Results: all the HCMV strains employed resulted in productive replication in ACC cells, as demonstrated by expression of both early (pp72) and late (pp65) viral antigens. Analysis of the kinetics of viral replication in ACC cells showed efficient production of infectious viral particles. HCMV infection of NCI-H295R cells significantly increased the production of cortisol and 17β-estradiol, an effect which was probably due to viral replication/viral gene expression rather than interaction of virus particles with the host cell, as demonstrated by experiments with UV-inactivated virus. This effect on steroid hormone production was accompanied by a marked induction of the storoidogenic enzymes CYP11B1 and CYP19. Moreover, HCMV infection showed a cytopathic effect on adrenocortical cells outlined by induction of apoptosis, cell vacuolisation, inhibition of cell proliferation, and increase of cellular S phase. Finally, evaluation of global human gene expression profile by microarray analysis showed that HCMV infection induced genes involved in growth control and stress response. Bayesian analysis unveiled two key genes, P2Y11 and P2X4, induced by HCMV infection and involved in adrenal steroidogenesis.
Conclusion: these results demonstrate for the first time that HCMV infects and replicates in human adrenocortical cells. HCMV infection of adrenocortical cells stimulates cortisol and estrogen production and changes cellular gene expression profile toward a condition of stress response.

P774 Evaluation of the AcroMetrix® OptiChallenge Inhibition Panel in different QIAGEN® sample preparation protocols automated on the QIAcube

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Objectives: The Clinical and Laboratory Standards Institute recommends that all clinical assay performances should be evaluated in the presence of potentially interfering substances to validate assays, to quantify interference effects, and to confirm interference in patient samples (CLSI EP-7-A2). This is particularly important for homebrew assays, where no data is available from the manufacturer on the performance of the assay in the presence of such substances. Clinical laboratories are responsible for verifying in all assays that the levels of interfering substances in their patient population are within an acceptable range.
Methods: The AcroMetrix OptiChallenge Inhibition Panel is a 7-member panel that mimics patient plasma containing potentially interfering substances that frequently occur due to clinical conditions such as hemolysis, icterus, or lipaemia.
We have evaluated the interference effect of the inhibition panel members on the performance of different silica-based sample preparation protocols on the QIAcube and their manual reference methods. Common bloodborne viruses, such as HCV and HBV, and different internal controls were spiked into each panel member in equal quantities.
Results: All tested automated sample-preparation protocols tested performed well and removed a good amount of the interfering substances when specific amounts of each analyte spiked were quantified in the presence of potentially interfering substances and compared with no-interference control. Results of the automated protocols were comparable to the manual reference methods. Different extraction and amplification chemistries show different susceptibilities to single interfering substances.
Conclusion: The automated QIAGEN extraction protocols, tested for isolation of viral RNA and DNA, have been proven to serve as reliable and robust methods. The inhibition panel was only minimally diluted, so the extraction systems were tested at the highest possible level of interference. The tested protocols were able to remove the majority of potentially interfering substances.
The OptiChallenge Inhibition Panel provides an effective tool to monitor the performance of nucleic acid extraction and amplification methods and simplifies the required validation processes by providing plasma samples that mimic interfering patient samples. This panel could be useful in QC release testing of DNA and RNA extraction and amplification systems.

P775 Importance of molecular detection for diagnosis and monitoring mumps-virus infection

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Objectives: Mumps-virus (MVs) is a paramyxovirus that causes fever, headache and swelling of the salivary glands. Complications such as meningitis, encephalitis and orchitis are relatively frequent, however permanent sequelae are rare. Routine vaccination included in immunisation schedule has resulted in a dramatic drop in the number of reported mumps cases, although, outbreaks in vaccinated population have recently occurred, including large outbreaks worldwide. World Health Organization (WHO) recommendations for diagnosis of mumps infection includes isolation of MVs from clinical specimens or seroconversion (or significant rise in serum mumps IgG titre) or positive serological test for mumps-specific IgM antibodies. These recommendations do not include molecular detection using RT-PCR. Our goal was to evaluate the importance of RT-PCR detection methods for MVs detection in clinical samples.
Methods: For that reason, we developed an study including serological and RT-PCR detection methods, in which 593 complete cases were analysed (complete cases include serum and saliva and/or urine samples).
Results: From these analysed samples, 296 (49.9%) were positive, being 30.7% IgM and RT-PCR positive, 29.7% IgM positive but RT-PCR negative and a surprising 39.5% IgM negative but RT-PCR positive. This 39.5% positive to RT-PCR showed 97.43% IgG positive, indicating a secondary vaccine failure since reinfection was discarded after analysis of patients vaccination antecedents.
Conclusions: Data presented in this study demonstrate the importance of molecular detection methods for diagnosis of MVs infection and for the evaluation of vaccine failure previously reported, since using only serological methods the MVs infection incidence is understimated.

P776 Serological and molecular diagnosis of measles using oral fluid samples

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Objectives: Measles Virus (MV) is responsible for a very contagious disease characterised by high fever, coryza, cough, conjunctivitis followed by the appearance of a maculopapular rash. Despite the development of a combined vaccine (Measles-Mumps-Rubella), measles remains a major cause of mortality in developing countries and a cause of continuous outbreaks in more industrialised countries. Currently measles is diagnosed by traditional serological and molecular assays on serum and nasopharyngeal secretions.
The introduction of oral fluid as an alternative medium to serum would open many perspectives. In advantage to venepuncture, the collection of oral fluid is less invasive, less painful, less expensive (i.e. no trained personal required) and safer (prevention of needle stick injuries). Since measles appears mostly in children a non invasive sample collection like oral fluid would be an enormous asset to determine measles RNA and antibodies as quick as possible in case of/or to avoid epidemic measles outbreaks.
Methods: In this study seventy-three measles positive and fifty measles negative tripled samples (serum, oral fluid and nasopharyngeal secretions) were analysed. Samples were collected in the Democratic Republic of Congo (Kinshasa) by the National Measles Laboratory. In addition 22/50 negative samples came from volunteers of the Scientific Institute of Public Health, Belgium (IPH). Oral fluid samples were collected using an Oracol collection device (Malvern Medical Developments) and were controlled for quality and quantity by an IgG quantification assay.

The detection of measles antibodies was performed by commercialised ELISAs. An in-house nested RT-PCR has been developed for the detection of measles RNA.

Results: The anti-measles IgM ELISA (MicroImmune) on oral fluid has been validated against the IgM ELISA (Dade Behring) on serum (=golden standard) and resulted in a sensitivity and specificity of respectively 93.1% and 100%. The validation of a molecular nested RT-PCR on oral fluid was performed against the standard assay on nasopharyngeal secretions and gave a sensitivity and specificity of 100%.

Conclusion: The obtained results confirm that both serological and molecular oral fluid assays are suitable for routine use.

The use of oral fluid samples may also promote the participation rate of patients, GP's and paediatricians in the Belgium measles surveillance system and other epidemiological studies in the framework of WHO elimination programme.

P777 Should PCR replace the indirect fluorescence antigen test for detection of Varicella zoster on skin swabs?

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Background: An indirect fluorescence antigen (IFA) test is classically performed for routine diagnosis of varicella zoster (VZV) infection on skin swabs. In this study the results of the IFA tests were compared to the results of PCR on skin swabs collected between January 2003 and June 2007.

Methods: Skin swabs were collected and immediately transported to the lab in virus transport medium (VTM). DNA extraction was performed on 200 μl VTM with a Qiagen blood kit (Qia). From august 2006 on, DNA extraction was performed with a NucliSens easyMAG extractor (EM) (bioMérieux). Elution volumes were 50 μl for Qia and 55 μl for EM.

PCR was performed according to the assay of Griffais et al. (NAR, 1991). From Sept 2005 on, this assay was replaced by a real-time PCR assay described by Stöcher et al (J.Clin. Virol. 2003).

The IFA test was performed by spotting the swab on a multispot plate. After addition of a MAb against VZV followed by incubation with a FITC labeled conjugate (Argene), the spots were analysed with a fluorescence microscope. The test was interpreted as positive, negative or not interpretable if the number of skin cells in the sample was too low.

Results: Between January 2003 and June 2007 a total of 380 swabs were analysed with IFA. A total of 283 swabs were scored as not interpretable (74.5%). Sixty one swabs were scored as negative (16%) and 36 as positive (9.5%). In total, 352/380 swabs were also analysed by PCR. In the IFA non-interpretable group, 273/283 were analysed by PCR. Of these, 75 were positive (27.5%) and 198 were negative (72.5%). Thirty four of the 36 IFA positive swabs were analysed by PCR. All were confirmed by PCR. In the IFA negative group, 45/61 were analysed by PCR. Three swabs were PCR positive and 42 were negative.

Conclusions: In this study, the performance of PCR was clearly superior to the IFA test that is routinely used for diagnosis of VZV infection on skin swabs. Especially, the number of uninterpretable results with IFA is unacceptably high. In this category, more than a quarter of the swabs produced a positive result by PCR, increasing the number of positive results as compared by IFA alone by more than 200%. Moreover, some additional positives were found in the IFA negative swabs. In Belgium, the IFA test is reimbursed by the national health ensurance system, while PCR on skin swabs is not. Therefore, switching the reimbursement to the most performing test should be considered.

P778 Evaluation of a new VIDAS® test for the detection of Epstein-Barr virus VCA IgG & IgM and EBNA IgG antibodies in human serum samples

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Objective: The aim of the study was to evaluate the performance of the 3 new automated VIDAS Epstein-Barr virus (EBV) reagents for the determination of the patient EBV serological characterised. The EBV reagents currently under development on VIDAS instrument are aimed to detect immunoglobulins against Viral Capside Antigen (VCA) and Epstein-Barr nuclear antigen (EBNA):VIDAS VCA IgG, VIDAS VCA IgM and VIDAS EBNA IgG

A comparison of the VIDAS EBV® tests was performed with EBV automated tests on the LIAISON® system (DiaSorin, Italy) using characterised sera samples.

Methods: VIDAS EBV VCA IgG and EBNA IgG reagents use specific EBV peptides coated on the solid phase to capture viral antigen immunoglobulins. The peptide-EBV IgG antibody complexes are later revealed using an anti-human IgG conjugated to Alkalin Phosphatase.

VIDAS EBV VCA IgM is based on immunocapture of serum EBV IgM. Twenty four sera from EBV primary infection, 20 sera from EBV past infection and 31 negative sera samples with a clinical status determined with the Immunofluorescence Gold Standard method (IF) were tested with the 3 VIDAS EBV markers to determine the performance. A concordance analysis between VIDAS and LIAISON was performed on an average of 75 samples on the 3 reagents. Discrepant results were analysed using IF.

Results: On defined IF characterised samples, the specificity of VIDAS VCA IgM, VCA IgG and EBNA IgG were 96.1%, 96.4% and 100% respectively and the sensitivity of VIDAS VCA IgM, VCA IgG and EBNA IgG were 100%, 95.6% and 100% respectively. For comparison, the specificity of LIAISON VCA IgM, VCA IgG and EBNA IgG were 98.0%, 96.4% and 97.6% respectively and the sensitivity of LIAISON VCA IgM, VCA IgG and EBNA IgG were 100%, 91.1% and 100% respectively.

Therefore, on this preliminary study VIDAS EBV overall performance leads to a more accurate clinical diagnosis of EBV primary infection than LIAISON EBV.

On random samples the concordance between VIDAS and Liaison VCA IgG, VCA IgM and EBNA IgG is 97.3%, 97.3% and 98.6%, respectively.

Conclusion: The new VIDAS EBV reagents presented good performance in accordance with the patients' serological status established with the IF. Evaluation on routine samples has also shown that VIDAS EBV reagents performed better than another automated test. VIDAS EBV reagents will be a good alternative to currently used manual method for MNI determination enabling single testing as well as series.

P779 Performance evaluation of VIDIA® CMV IgG assay, a new automated immunoassay for the detection and quantification of CMV IgG in human serum and plasma

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Objective: The VIDIA system is a new automated, primary tube immunoassay instrument designed to reinforce traceability and simplify the daily workload for routine testing.

We evaluated the performance of VIDIA CMV IgG (bioMérieux) in terms of measuring range, precision, sensitivity, specificity. A correlation study with VIDAS CMV IgG was also performed.

Material and Methods: The VIDIA CMV IgG assay principle combines a two-step "ELISA indirect" immunoassay method with a final chemiluminescence detection. In the first step CMV IgG antibodies present in the sample bind with purified CMV Ag coated on the magnetic particles and with the anti-human IgG monoclonal conjugated with alkalin phosphatase (PAL) for the revelation phase.

The results of the tests are expressed in AU/ml (Arbitrary Unit). The positive cut-off was defined at 3 AU/ml, a grey zone is defined between 2 and 3 AU/ml and samples below 2 AU/ml are considered as negative. The study was performed by testing 60 positive and 43 negative samples. The serological status of the samples was established according to the results obtained with 3 CE-marked immunoassays: VIDAS CMV IgG (bioMérieux, France), CMV-IgG-ELA Test PKS medac (medac Diagnostika Germany), Enzygnost Anti CMV/IgG (Dade-Berhing, Germany).

Correlation with VIDAS CMV IgG was performed on 291 samples.

The study was done in the bioMérieux R&D laboratory during the development phase of the VIDIA CMV IgG reagent.
Results: With a measuring range up to 400 AU/ml, the precision obtained for samples at 10 AU/ml up to 250 AU/ml was between 4% and 12%, respectively.
Sensitivity and specificity were 100% on 103 samples tested.
Correlation coefficient between VIDIA CMV IgG and VIDAS CMV IgG was found at 0.89 and the concordance between the results of the 2 tests was 100%.
Conclusion: The very good concordance found between VIDIA CMV IgG and the status of the samples defined 3 CE-Marked tests, leads to a 100% sensitivity and specificity for the new VIDIA test under development. VIDIA CMV IgG is also well correlated with VIDAS CMV IgG and presents a good precision throughout the whole measuring range.
These first results show that VIDIA CMV IgG could adequately discriminate between IgG positive and negative samples, even if these results will have to be confirmed on the final commercialised product.

P780 Rapid and efficient detection of viral gastroenteritis by an automated multiplex real-time PCR approach

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Objectives: Traditionally, viral gastroenteritis is diagnosed by antigen detection, electron microscopy, and increasingly PCR. However, this necessitates the use of separate assays for the different viruses. Multiplex real-time PCR (MPCR) provides an efficient alternative for diagnosis of viral gastroenteritis, especially when limited specimen is available In addition, it may be complemented by a similar approach for bacteria and parasites. In this study we compared our traditional detection methods to a 3-tube multiplex real-time PCR.
Methods: Stool specimens (n = 212) were obtained between July and December 2007 for routine diagnostics of gastrointestinal viruses at UMCG. Routine procedures consisted of antigen detection (astrovirus [hAsV], rotavirus [hRoV]), or singleplex real-time PCR (adenovirus [hAdV], enterovirus [hEV], norovirus genogroup I + II [hNoV], parechovirus [hPEV]). Nucleic acids were isolated by NucliSENS easyMAG specific A protocol (bioMérieux) using the phocin distemper virus (PDV) as internal control. Multiplex real-time PCRs included hAsV, hNoV, hRoV, PDV (Mplex 1), hEV, hPEV, PDV (Mplex 2), and hAdV, bocavirus (hBoV) (Mplex 3). Detection of hBoV was included to study its possible role in gastroenteritis.
Results: Fully concordant results were obtained by MPCR in 96.3% of tests requested. Positive specimens not detected by MPCR were exclusively specimens with low levels of viral RNA (Ct > 39), except for 1 clearly antigen positive hRoV. MPCR detected more hRoV (4/153), hPEV (8/185), and hAsV (1/135) in specimens negative by routine procedures. In addition, MPCR detected 3 positive hRoV infections in specimens with insufficient volume for antigen testing. Another 30/212 specimens tested positive for hAdV, and 7/212 for hBoV, although in the latter case, Ct values were always >35. Unresolved results due to inhibition were obtained for 3, 4, 6, and 3 specimens by routine PCR, Mplex1, Mplex2, and Mplex3 respectively.
Conclusion: MPCR provides a feasible alternative to separate routine diagnostics tests for detection of viral gastroenteritis. MPCR increased the sensitivity for hRoV and hPEV, although the sensitivity for hEV was slightly reduced. However, the clinical relevance of the infections missed by MPCR seems limited based on the high Ct values. Finally, the role of hBoV in the aetiology of viral gastroenteritis seems questionable based on the amount of hBoV DNA detected.

P781 Comparative evaluation of the NucliSENS easyMAG automated system for the extraction of viral DNA from whole blood samples: application to the monitoring of cytomegalovirus and Epstein-Barr virus loads

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Objectives: Due to the high amount of genetic material in whole blood samples, the new extraction methods must be carefully evaluated by comparison to the reference techniques such as those using columns to recover correctly the nucleic acids used in amplification techniques. The aim of this study was to evaluate the ability of the NucliSENS easyMAG automated system (bioMérieux) using magnetic silica to extract viral nucleic acids from human whole blood for the determination of the viral load in patients suspected of cytomegalovirus (CMV) or Epstein-Barr virus (EBV) infection.
Methods: Two hundred microlitres of whole blood were extracted either by the manual Qiagen method (QIAamp DNA blood extraction kit) or by the "Specific B" protocol on the NucliSENS easyMAG instrument. CMV or EBV DNA was quantified by using the respective R-gene amplification kits (Argene). Ninety six samples (including 75 found initially positive in our laboratory) and 80 (59 positive) were selected for CMV and EBV analysis, respectively.
Results: A total agreement between the two extraction techniques was of 88.5% for CMV and 81.3% for EBV. The sensitivity of the NucliSENS easyMAG extraction method was 89.3% and 100% for CMV and EBV respectively; for comparison Qiagen extraction method exhibited a sensitivity of 82.6% and 83.1% for CMV and EBV respectively. As EBV tests, CMV tests were performed from same samples to compare the two extraction methods: the sensitivity difference with the conventional method used in our lab could be attributed to samples storage. For samples found positive after extraction by both methods, the correlation coefficient (r test) between the viral loads was 0.855 for CMV and 0.942 for EBV; the mean between the difference in viral loads was –0.00859 log copies/mL for CMV (not statistically significant) and 0.22 log copies/mL for EBV to the benefit of the automated extraction method ($p < 0.001$).
Conclusion: The excellent concordance between the results obtained after using both extraction methods validates the capacity of the "Specific B" protocol to extract viral DNA from whole blood with the NucliSENS easyMAG system in two models measuring viral load. A trend was noted for a better sensitivity of NucliSENS easyMAG regarding the low values. The additional advantages of this automated extraction technique include: reduction of technical time (2/3) and of cross-contamination avoiding centrifugation, and improvement of standardisation, traceability and quality control assessment.

P782 Evaluation of a new immunochromatography test for the detection of Norovirus

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Objectives: Norovirus is one of the most common pathogens causing gastroenteritis and belongs to the family Caliciviridae, which is a non-enveloped, positive-sense, single-stranded RNA virus. RT-PCR and ELISA are widely used detection methods, which have drawbacks such as long turnaround times, relatively high cost, need for specialised equipment and trained technicians. The newly developed Quick Ex-Norovirus "SEIKEN" assay is based on immunochromatography (IC) method, which is easier to perform, rapid, cost efficient and offers true clinical value.
Materials and Methods: A total of 163 faecal specimens obtained from adults, children and infants in Japan, who showed acute gastroenteritis symptoms during period May 2006 to March 2007, were collected and stored in a public laboratory and two hospitals in Japan.
The reactivity of this test was evaluated against Virus-like particles (VLPs), containing 6 genotypes of genogroup 1 (G1) and 13 genotypes of genogroup 2 (GII).

The test was performed according to the instruction manual. In summary, 0.1 g of faecal specimen (or 100 μL of VLPs) was added to the specimen preparation tube, containing 0.9 mL of specimen buffer, then mixed with a tube mixer for 30 sec. and centrifuged at 2000xg for 5 min. An aliquot of 300 μL of supernatant was transferred to a sample tube and after capping this tube with a specimen filter, filtrate is collected into a reaction tube. The IC test strip was inserted into this tube and interpretation was done after 15 min. incubation time at room temp. All clinical specimens were also tested by RT-PCR, as described by Yan et al. (2003). A primer set of G1-SKF and G1-SKR was chosen for G1 and a set of G2-GKF and G2-SKR for GII identification.
Results: Fifty three (53) of 163 samples (32.5%) were positive by the IC test. Sensitivity, specificity and overall agreement were 73.6%, 98.9% and 87.7% respectively, when compared with RT-PCR. Nineteen (19) specimens were negative by the IC test, but positive by RT-PCR. These discrepancies might be due to low viral load in these specimens. The IC test showed positive against all VLPs, including GI types 1, 2, 3, 4 & 6 and GII types 1 to 8, 12 to 15 and 17.
Conclusion: The sensitivity and specificity of the IC test for clinical specimens were comparable with current Norovirus detection methods, hence the Quick Ex-Norovirus "SEIKEN" assay might be a valuable attribute in the clinical setting.

HIV – Therapeutics

P783 Neoplasia in patients with HIV infection: effect of HAART

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Surveillance data indicate that, the incidence and type of HIV related neoplasias has changed after the introduction of HAART.
Objective: To determine the incidence and types of cancers in the pre-HAART and post-HAART eras, and the differences between women and men.
Methods: Retrospective record review of HIV infected patients with cancer from January 1991 to December 2003 at a teaching hospital.
Results: A total of 268 HIV patients with cancer were identified, 206 men (age: 38±11 years, CD4: 123±39/μL) and 62 women (age: 44±16 years, CD4: 68±27/μL), of them 90 were detected in the pre-HAART (33.6%) and 178 in the post-HAART era (66.4%, p=n/s). AIDS defining cancers (Total: 215, 72.4%; of them 108, 77.7% in men vs. 31, 22.3% in women) were more frequent pre-HAART (Total: 76, 54.7%; of them 60, 43% in men vs. 16, 11.5% in women) than post-HAART (Total: 63, 45.3%; of them 48, 34.5% in men vs. 15, 10.8% in women). Non AIDS defining cancers (Total: 53, 27.6%; of them 34, 64% in men vs. 19, 36% in women) were less frequent pre-HAART (Total: 14, 26.4%; of them 9, 17% in men vs. 5, 5.3% in women) than post-HAART (Total: 39, 73.6%; of them 25, 47.2% in men vs. 14, 26.4% in women). Total cancer related mortality was higher pre-HAART (61% vs 52%, $p < 0.05$) and in women (60% vs 55%, p=n/s) than post-HAART (Total: 53, 27.6%; of them 36, 18.8% in men vs. 17, 8.9% in women). AIDS defining cancer related mortality was higher pre-HAART (65.8% vs 36.5%, $p < 0.01$) in both sexes ($p < 0.01$), while non AIDS defining cancer related mortality was higher post-HAART (76.9% vs 35.7%, $p < 0.01$) in both sexes ($p < 0.01$).
Conclusions: The incidence of non AIDS defining cancer increased, although the total incidence of HIV related neoplasia has not changed after HAART. Total cancer related mortality is higher pre-HAART and in women. Both sexes have a higher mortality by AIDS defining cancer pre-HAART and, by non AIDS defining cancer post-HAART.

P784 Influence of anti-retroviral treatment on gastric secretion, *Helicobacter pylori* colonisation of gastric mucosa and fungal colonisation of upper gastrointestinal tract in HIV-infected patients

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Objectives: HIV infection is considered to have negative influence on gastric acid juice secretion and results in higher prevalence of fungal colonisation of upper gastrointestinal (GI) tract, whereas *Helicobacter pylori* (Hp) prevalence in stomach seems to be lower in HIV-infected patients (pts). The aim of the study was to estimate influence of antiretroviral treatment (HAART) on gastric secretion, Hp colonisation of stomach and fungal colonisation of upper GI tract.
Methods: 94 HIV-infected pts were studied, half of them had CD4 lymphocyte count <200/uL. 9 of 21 pts receiving HAART belonged to this group. Biopsy samples were taken during endoscopy from corpus and antrum of stomach to confirm presence of Hp by histological analysis and rapid urease test. Quantitative mycologic cultures from oral and oesophageal swabs were analysed in 70 pts. Gastric secretion was evaluated in 37 pts using Kay method on the basis of BAO, MAO and PAO. The results were compared to HIV viral load (VL) detected by PCR.
Results: VL decrease correlated with increased BAO (correlation coefficient r=-0.165), MAO (r=-0.287) and PAO (r=-0.289) and decreased BAO/MAO ratio (r=0.22). Prevalence of Hp in pts on HAART in comparison to the rest of pts, was lower in both corpus of stomach (28%vs48%) and antrum (38%vs62%) /$p > 0.05$/. Hp was present in corpus and antrum (respectively) in 29%/47% of pts with VL < 500 copies/mL and 44%/49% of pts with higher VL /$p > 0.05$/. Fungal colonisation rate in pts on HAART (vs the rest of pts) was lower in both oral cavity (64%vs87%) and oesophagus (64%vs84%) /$p > 0.05$/. Lower prevalence of fungal colonisation in pts with VL < 500 copies/mL (vs the rest of pts) was found in oral cavity (67%vs90%) /$p > 0.05$/ and oesophagus (58%vs87%) /$p < 0.05$/.
Conclusions: Inhibited HIV replication due to antiretroviral therapy results in increase in gastric secretion, as well as decrease in fungal colonisation of the upper GI tract and Hp colonisation of stomach.

P785 Liver toxicity and plasma drug levels of lopinavir in HIV/HCV co-infected patients according to the degree of liver fibrosis

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Objectives: HCV co-infection is a well known risk factor for developing hepatotoxicity in HIV-infected patients receiving HAART. However, few data exist about liver toxicity with r/lopinavir and the influence of plasma drugs levels, especially in patients with different stages of HCV disease.
Methods: Prospective cohort study of 51 HIV/HCV co-infected patients who underwent a liver biopsy and who received lopinavir as the third drug regimen. Liver fibrosis was staged using a scoring system of 0 (no fibrosis) to 4 (cirrhosis). Hepatotoxicity was defined as an increase in AST/ALT levels over five times the upper limit of normal, or a 3.5-fold increase if baseline levels were abnormal, with or without clinical symptoms of liver toxicity. Plasma through levels of lopinavir were determinated by HPLC in all the cases.
Results: Mean age was 41 yrs, 73% were male, and 73% were former IDUs. Of note, 58% of patients had a prior diagnosis of AIDS. Mean estimated time of HCV infection was 17.6 yrs, mean HCV viral load was 1.298235 UI/ml, and the predominant HCV genotypes were 1 and 3. Median plasma drug levels of lopinavir were 7315 ng/ml (638–11206), with differences according to the stage of fibrosis (8114, 6614, 6008, and 4412 ng/ml for fibrosis stage 1 to 4, respectively). Notably, in three cirrhotic patients with an advanced stage of disease (MELD scale >11), median plasma through levels were 881 ng/ml. During an accumulated time of 461 months on r/lopinavir therapy, there were 5 cases of hepatotoxicity. In these patients, plasma trhough levels of lopinavir were

similar to those observed in patients not developing toxicity (6502 vs 7368 ng/ml).
Conclusions: The risk of liver toxicity was very low in HIV/HCV co-infected patients receiving r/lopinavir. In these patients, plasma drug levels of lopinavir showed high interpatient variability and were not associated with toxicity.

P786 Dream changes after efavirenz treatment

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Objective: Although sleep disturbance is a frequent side effect of efavirenz, there is no studies about abnormalities in the quality of dreams.
Method: This is a pilot study. HIV patients without neuropsychiatric diseases underwent a polisomnography (PSG) study before and after efavirenz treatment. Efavirenz plasma levels were assessed before the second (PSG). Patients were awoke at first REM sleep period during the night and at REM sleep period occurring after 5:00 am and invited to tell the dream. Dream changes were evaluated by recall rate, number of thematic units and emotional content classified into four categories: violent/highly anxious, moderately anxious, pleasant and neutral. Dream length was assessed by the number of words included in the description. Chi square, Student T test and Z signs test were used to compared results.
Results: Eighteen PSG were performed in ten patients. Second PSG was performed after 10.4 (SD 5.4) days of efavirenz treatment with all patients having therapeutic efavirenz plasma leves (>1 μg/ml). Dreams were recalled in 84% before efavirenz and 43% after efavirenz (p = 0.024). There were no differences in the mean number of words per dream before and after efavirenz treatment (61.96 versus 47.5, p = 0.115) neither in the number of thematic units (16 versus 6, p = 0.442). There were less anxious and violent dreams after efavirenz (3 versus 1). The proportion of dreams with no neutral emotional content (either pleasant or unpleasant) was 37.5% before efavirenz and 66.7% after efavirenz treatment (p = 0.046).
Conclusions: Efavirenz treatment increases the proportion of no neutral dreams in this group of patients. There were no longer dreams and no more dreams with negative emotional content after efavirenz treatment. Dream recall was lower after efavirenz treatment. Further studies in this area are needed.

P787 Improved long-term survival among AIDS patients with progressive multifocal leukoencephalopathy receiving potent antiretroviral therapies

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Objective: To determine the outcome of progressive multifocal leukoencephalopathy (PML) in patients with AIDS.
Methods: A retrospective cohort of 52 subjects with AIDS who were diagnosed of PML (clinical and neuroradiological findings of active PML, no other aetiology, and/or PCR detection of JCV DNA in CSF, and/or histopathological findings in biopsy) from 1996 to 2007.
Results: At diagnosis, the median CD4+ cell count and plasma HIV RNA level were 93/mL (range, 7–410/mL) and 4.8 log copies/mL (2–5.7), respectively. A probable immune reconstitution inflammatory syndrome (IRIS), defined as new onset (<180 days) of symptoms shortly after initiation of HAART, was described in 10 cases (19%). As expected, patients with IRIS had higher CD4+ count (181 vs 117/mL) and a lower HIV RNA level (2.95 vs 4.6 log copies/mL, p = 0.002) at the time of diagnosis. JCV DNA was detected in 52% of cases, and, of note, the CD4+ count was higher in patients presenting with a negative value (156 vs 113/mL). Moreover, JCV in CSF was mostly detected in patients without IRIS (57 vs 33%). During a median follow-up of 745 days, a total of 14 patients died (27%), most of them during the first 3 months after diagnosis (13 out of 14 cases, 93%). The probability of survival was 90% and 70% at 24 weeks for patients with or without IRIS, respectively (p = 0.15, log-rank test). There was no association between initial CD4+ count and mortality (28% vs 25% for patients below or above a CD4+ count of 100/mL). At the end of follow-up, HIV viral load was <50 copies/ml in 64% of cases, and CD4+ cell count was 357/mL, without differences according to initial response.
Conclusions: The prognosis of PML in patients with AIDS receiving potent antiretroviral therapies has improved significantly, and, for patients surviving three months after the diagnosis, the outcome is similar to that observed in the HIV population. IRIS diagnosis was not associated with a worse outcome.

P788 Safety and efficacy of a fixed-dose combination of zidovudine, lamivudine, and nevirapine in antiretroviral-naive and –experienced HIV-1 infected patients

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Objectives: To evaluate the safety and efficacy of a fixed-dose combination (FDC) of zidovudine, lamivudine, and nevirapine (AZT/3TC/NVP) which has recently been available in developing countries and is planned to replace the extensive use of the FDC of stavudine, lamivudine, and nevirapine (d4T/3TC/NVP).
Methods: A cohort study was conducted in HIV-1 infected patients who were initiated FDC of AZT/3TC/NVP as an initial antiretroviral therapy (ART) or as switching ART from other stable regimens at a medical-school hospital in 2006. Patients were followed up for 12 months and were categorised into initial ART and switching ART groups.
Results: A total of 241 patients were included; 62% were male and mean (SD) age was 40.4 (8.7) years. Mean (SD) body weight and hemoglobin were 60.2 (11.3) kg and 13.4 (1.8) g/dl, respectively. Of 241 patients, 51 were in initial ART group and 190 were in switching ART group. In initial ART group, median (IQR) baseline CD4 cell count and plasma HIV-1 RNA were 159 (70–210) cells/mm^3 and 5.4 (4.6–5.9) log copies/ml, respectively. In switching ART group, median (IQR) baseline CD4 cell count was 386 (276–519) cells/mm^3 and all had plasma HIV-1 RNA <50 copies/ml. Of 190 patients, 184 (97%) switched from NNRTI-based regimens and 116 (61%) were FDC of d4T/3TC/NVP. Median (IQR) duration of ART prior to switching was 34 (22–51) months. By intend-to-treat analysis, 78% in initial ART group and 91% in switching ART group had plasma HIV-1 RNA <50 copies/ml at 12 months of FDC of AZT/3TC/NVP. At 6 and 12 months, median CD4 cell count significantly increased from baseline to 303 (p =0.030) and 352 (p < 0.001) cells/mm^3 in initial ART group; 415 (p = 0.026) and 436 (p = 0.018) cells/mm^3 in switching ART group. During 12-month follow-up period, 14% and 6% of patients in initial ART group and switching ART group, respectively, had discontinued FDC of AZT/3TC/NVP due to adverse events. These adverse events (initial ART, switching ART) included anaemia (4%, 3%), rashes (6%, 0.5%), nausea/vomiting (4%, 1%), hepatitis (2%, 0%), myalgia (0%, 0.5%), and non-improved lipodystrophy (0%, 0.5%).
Conclusions: A FDC of AZT/3TC/NVP has good safety and efficacy for using as initial ART in antiretroviral-naive patients and for switching ART in patients with stable ART and complete viral suppression. The virological and immunological outcomes are favourable. In resource-limited settings, a FDC of AZT/3TC/NVP is an alternative for replacing a FDC of d4T/3TC/NVP.

P789 Tolerance and quality of life improvements: Kaletra® soft-gel capsules vs Kaletra® tablets: the KaleVie study

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Background: Kaletra® tablets were introduced in september 2006 in France to replace soft gel capsules (SGC) with no need for refrigeration or dosing with food, and a new excipients formulation. The aim of this study was to evaluate in real life the tolerance, the change of comfort of drug intake, and quality of life between the end of the last month on SGC (M0) and the first month on tablets (M1).

Methods: From september 2006 to february 2007, an open investigation, multicentre prospective study was conducted on HIV patients (pts) switching from Kaletra® SGC to tablets to compare the course of comfort of drug intake, quality of life, confidentiality, with or without food intake, preservation of drug, tolerance, and impact of new tablet formulation on everydaylife between M0 and M1.

Results: 81 pts were included (59 males, 22 females); mean age: 44 (29–77 years). Seropositivity detections dated back to 11 years (1 month – 21 years). Mean duration of antiretroviral treatment was 9 years: 2 years on Kaletra® (1 month – 6 years).

Comfort of drug intake evaluated on a 0 to 10 graduated scale went up from 7.9 to 8.6 ($p < 0.05$), and 73% at M1 compared to 64% at M0 did not have quality of life affected with HIV infection and treatment. Removing food restrictions was very important for 50% of pts; no need for refrigerate upgraded confidentiality for 65% of pts and was considered as very important for 94%. Food intake with SGC or tablets increased gastro-intestinal tolerance in 75% pts. Diarrhoeas and gas were less frequent and severe when switching to tablets ($p < 0.05$) and were not more associated with a regimen including AZT ($p > 0.05$) or a first month of Kaletra© treatment ($p > 0.05$). There was less dislike with the yellow tablets than orange SGC ($p < 0.05$). Observance did not increase ($p > 0.05$) but no patient encountered any confidentiality problem leading to miss an intake and 23% had the feeling tablets were more efficient; 18% declared they would change their travel habits at M1.

Conclusion: Following switch from SGC to tablets, subjects reported an increased quality of life and confidentiality, and a better tolerance. Food intake lowered gastro-intestinal adverse events with either SGC or tablet formulation.

P790 Efficacy and safety of filgrastim in HIV/AIDS patients with bacterial infections

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Background: To assess the effect of filgrastim (G-CSF) in HIV/AIDS patients with bacterial infections with or without neutropenia since it is supposed that filgrastim, together with increase of neutrophil count, can also enhance their functional activity and thus improve outcome of bacterial disease.

Methods: 48 HIV/AIDS patients with severe bacterial infection/sepsis aged 19–60 (study group) were enrolled in the randomised controlled, open-label, prospective follow-up study. Among them 29 patients were neutropenic with absolute neutrophil count (ANC) $<1.0\times10^9$/l, and 19 were non-neutropenic. Patients in the study group were treated with standard therapy (antimicrobial drugs and symptomatic treatment) and filgrastim 5 μg/kg daily for 5–7 days. In control group were included 17 HIV patients with severe bacterial infection or sepsis with (9 patients) or without (8 patients) neutropenia on standard therapy without filgrastim. Functional activity of neutrophils was measured by nitroblue tetrazolium reduction (NBT)-test.

Results: As neutropenic so non-neutropenic filgrastim-treated patients required fewer hospital days for infections and fewer days of intravenous antibacterials compared to control group; death and relapse rate was less frequent in patients with filgrastim. Severe neutropenia was less frequent in neutropenic patients with filgrastim compared to control patients. NBT-test showed improved neutrophil's functional activity in filgrastim-treated patients. Filgrastim was not associated with increase in HIV plasma RNA level or any new or unexpected adverse events.

Conclusions: Filgrastim was safe and effective for severe bacterial infection/sepsis in HIV/AIDS patients with or without neutropenia. Filgrastim was well tolerated by patients and significantly improved the patient's quality of live. It may reduce the duration of infections, hospital days for infections, days of intravenous antibacterial agents and risk of relapse. Filgrastim treatment was also associated in improved neutrophil functional activity.

Further studies are required to optimise treatment regimen.

P791 Pharmacokinetics of lopinavir combined with rifabutin in a clinical setting

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Background: The use of ritonavir-boosted lopinavir (LPV/r) plus adjusted dose rifabutin (aRFB) is currently allowed in HIV-infected subjects with mycobacterial disease (MD). This study investigated the pharmacokinetic (PK) parameters of LPV/r when associated with aRFB in a clinical setting, from which no such data have been published thus far.

Methods: This prospective, multicentre study enrolled consecutive HIV-infected subjects with MD for whom clinicians chose a combination regimen including LPV/r (standard dose)+aRFB (150mg 3x/week), associated with any anti-mycobacterial (AMB) drugs and no antiretroviral (ARV) drugs other than nucleos(t)ide reverse transcriptase inhibitors. Treatment adherence was supervised. A 12-hour PK assessment of plasma LPV levels was performed on ⩾1 occasion after >14 days of LPV/r+aRFB administration and, when feasible, after >14 days of RFB withdrawal. LPV plasma levels were centrally assayed by a validated HPLC-UV method (lower quantification limit 50ng/mL). PK parameters were calculated by standard non-compartmental methods (KINETICA, version 4, InnaPhase). Intra-subject changes in PK parameters were evaluated by calculating geometric mean ratios (GMR) and 95%CI (SAS).

Results: LPV PK parameters were obtained in 25 subjects during aRFB co-administration: median age 40 years (range 27–54), weight 61Kg (42–77), males 72%, Caucasian 64%, black 20%, other races 16%, HVC or HBV co-infection 32%, liver impairment 24%. MD was tubercular in 64%, non tubercular (24%) and undefined (12%). Mean±SD LPV Ctrough and Cmax were 11.3±5.1 and 18.7±6.9 μg/mL, respectively. AUC0–12h was 166.2±63.4 μg.h/mL and CL/F 0.05±0.02L/h/kg. LPV PK assessments in absence of RFB were also available for 6/25 patients. The GMR (95%CI) of LPV CL/F/Kg, Ctrough, Cmax and AUC0–12h when associated with aRFB versus alone were 1.17 (0.66–2.07), 0.84 (0.40–1.77), 0.80 (0.47–1.37) and 0.88 (0.44–1.76), respectively.

Conclusions: Mean LPV Ctrough and AUC0–12 during aRFB co-administration in our clinical population were well above the minimum recommended target levels (even those recommended for antiretroviral experienced patients). LPV showed a non-significant intra-patient mean increase in CL/F (17%) when associated with aRFB versus alone; however, this finding evidenced a high inter-subject variability, probably determined by the different patient characteristics which emphasizes the usefulness of therapeutic drug monitoring during AMB-ARV combination treatments.

Nosocomial outbreaks

P792 Cross-transmission of a multidrug-resistant *Acinetobacter baumannii* clone produced endemic nosocomial bacteraemia and one outbreak in a Mexican Hospital

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Background: Multidrug-resistant *Acinetobacter baumannii* (Aba) has emerged as an important cause of endemic nosocomial infections and epidemic outbreaks.

Objectives: To describe the antimicrobial susceptibility profile associated to genotyping of Aba causing nosocomial infections and outbreaks in one Mexican Hospital.

Methods: This is a descriptive molecular epidemiology study performed in a 1,000-bed tertiary care University Hospital in Guadalajara, Jalisco, Mexico. From December 2002 to August 2007, one hundred Aba isolates from nosocomial infections were detected from several wards in Hospital Civil de Guadalajara. Clinical data were collected from medical records. Antibiotic susceptibility testing was performed by disc diffusion method

according to CLSI guidelines. Clonal analysis was determined by ApaI – Pulsed Field Gel Electrophoresis (PFGE).

Results: Sites of isolation included: Skin and soft tissue (46%), bloodstream (42%), sterile fluids (8%), and respiratory tract (4%). All Aba isolates were resistant to all quinolones, aminoglycosides, and β-lactam antibiotics, with 50% resistance to carbapenems. Genetic fingerprinting by PFGE of these isolates showed extensive heterogeneity between isolates, although one major cluster involving 37 patients belonged to a single clonal type. This Aba clone was an endemic in 2005 and 2006, and produced an outbreak in 2007. This clone was detected in the majority of the hospital wards.

Conclusions: Proper control of this multidrug-resistant Aba will require a multidisciplinary approach, including identification of resistance mechanisms, optimising antibiotic use based on local epidemiology and vigorous implementation of infection control procedures.

P793 Spread of multidrug-resistant *Acinetobacter baumannii* in intubated patients

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Objectives: *Acinetobacter baumannii* constitutes one of the most common cause of ventilator associated pneumonia in intensive care units. The colonised patients are important source for environmental contamination and patient-to-patient spread of microorganism. The major mode of transmission from patient-to-patient and environment-to-patient is contaminated hands of healthcare workers. The aim of this study was to determine the risk factors for the colonisation of respiratory tract and infection with *A. baumannii* in medical ICU (MICU) and examine the genetic relatedness of the strains to show the spread of isolates.

Methods: This study was conducted prospectively between December 1, 2004 and January 31, 2006 in MICU. Patients (>16 years) admitted to the MICU and mechanical ventilated and/or intubated were enrolled in the study. Endotracheal aspirate of these patients were screened for *A. baumannii* at the beginning of intubation (in the first 48 hours), at the end of first week and at the time of withdrawal of endotracheal tube. Patients intubated shorter than 48 hours were not included into the study and data of *A. baumannii* colonised or infected patients at ICU admission were not evaluated for the risk factors. Strains isolated from colonised and infected patients were evaluated for genetic relatedness. Genotyping analysis was performed by pulsed-field gel electrophoresis (PFGE)

Results: Ninety-eight patients were evaluated for *A. baumannii* colonisation during or at the end of their entubation and 44 (45%) of these patients were colonised with *A. baumannii*. The mean time for *A. baumannii* acquisition was 7.46±3.74 days (range 2 to 16 days). The length of entubation (OR:1.032, p: 0.014) and diabetes mellitus (OR:4.140, p:0.008) were found to be the major risk factors for the colonisation of respiratory tract with *A. baumannii*. During the study period, *A. baumannii* infection developed in 35 (80%) of 44 colonised patients. The important risk factors for infection were *A. baumannii* colonisation (OR:3.962, p:0.006) and tracheostomy (OR:4.857, p:0.001). Genotyping analysis was performed for 59 isolates. Overall, three clones (clone A, B, C) were determined from patients, whereas 88% belonged to clone A (52 isolates), 7% clone B (four isolates) and 5% clone C (three isolates).

Conclusion: This study showed the spread of *A. baumannii* in intubated patients in a ICU with inadequate infrastructure.

P794 Risk factors for nosocomial *Acinetobacter* bacteraemia: a case-control study of intensive care unit patients

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Objectives: This study was performed to identify risk factors and to determine the attributable mortality and outcome of *Acinetobacter* bacteraemia in intensive care unit.

Methods: A retrospective case-control (1:1) study was conducted in a tertiary, academic hospital, with 300 beds. From January 2001 to December 2006, 54 cases and 54 controls were included. Cases were all the true nosocomial bacteraemias by *Acinetobacter* spp. A control was defined as the consecutive patient with negative blood cultures, matched by sex, age (±10 years), primary and secondary diagnosis, APACHE II score (±2 points), operative procedures, and date of admission. Characteristics and mortality rates of patients with *Acinetobacter* bacteraemia and their controls were compared. The results were analysed using SPSS (version 11.0) for Windows.

Results: The mean age was 51.05±22.30 years (median = 56) for the cases, and 47.90±20.58 years (median = 44) for the controls. There was a trend for a longer median duration of hospitalisation among case patients, compared with control patients (33.31±30.67 versus 11.55±10.50 days; p = 0.06). Patients with *Acinetobacter* bacteraemia had significantly more hemodynamic instability (hypoxia, shock) and longer length of ventilator requirement than controls. Case-patients were more likely than controls to have had central venous catheter, peripheral arterial catheter, mechanical ventilation and total parenteral nutrition ($p < 0.05$). Thirty-three (61.1%) of the case patients died, compared with 14 (25.9%) of the control patients (p = 0.001). Attributable mortality is determined by subtracting the crude mortality rate of the controls from the crude mortality rate of the cases. The attributable mortality was 35.2%.

Conclusion: In critically ill patients *Acinetobacter* bacteraemia is associated with a significantly increased mortality rate.

P795 Role of tigecycline in the control of outbreak of carbapenem-resistant *Acinetobacter baumannii* infections in an intensive care unit, Kuwait

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Objective: To report 3 different episodes of nosocomial outbreaks of multi-resistant *Acinetobacter baumannii* (MRAB) infections in an ICU setting, successfully controlled with tigecycline.

Methods: The outbreaks of *Acinetobacter* infections occurred in February and April, 2006 and April 2007. Patients' ages, nationality, underlying co-morbidities, prior antibiotics used, site of infection, major reason for hospital admission, whether or not ventilated and length of ICU stay, were recorded. Samples of endotracheal tube (ET) secretions, sputum, blood, urine, CSF and wound were collected and cultured on appropriate bacteriological media. The isolates were identified using automated VITEK 2 ID system and the API 20NE system. Susceptibility testing was done by the Etest method. Molecular typing of the isolates was determined by PFGE. Patient and environmental screening strategies previously agreed to by the Outbreak Management Committee were carried out. Patients were nursed in isolation rooms or single side rooms.

Results: A total 21 patients, aged 19–75 years, were involved. Time of admission to time of acquiring infection ranged from 3–31 days. All isolates were resistant to 15–17 antibiotics, including carbapenems (MRAB-C) but susceptibile to tigecycline with MIC90 of 2 μg/ml. Overall mortality rate was 14.3%. The first outbreak involved 6 cases with pnuemonia, meningitis and UTI. Time to clearance of the MRAB-C after therapy with amikacin and ciprofloxacin was 8.3 days and was associated with a mortality rate of 50%; tigecycline was unavailable for use at this time. The second outbreak involved 10 patients (6 bacteraemias, 3 pneumonia and 1 UTI). All patients received tigecycline in adequate doses. Time to clearance was 2.8 days and all survived. Five patients (3 bacteraemias, 1 pneumonia and 1 abscess), were involved in the third outbreak. They were also treated with tigecycline. Time to clearance was 3.1 days and all survived. Environmental screening revealed gross contamination of many surfaces and equipment within the unit. Genotypic characteristics demonstrated 2 distinct clonal strains. Other measures included closure of the ICU to new admissions during the second outbreak to allow for adequate cleaning and disinfection of the unit.

Conclusion: Two clones of MRAB-C were responsible for the outbreaks and successful control included the use of tigecycline and aggressive infection control strategies.

P796 An outbreak of multidrug-resistant *Acinetobacter baumannii* colonisation and infection in a neonatal intensive care unit of a major paediatric university hospital in Athens, Greece

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Objective: The aim of this study was to investigate and report the epidemiologic, bacteriologic and clinical features of an outbreak caused by *A. baumannii* in a Neonatal Intensive Care Unit (NICU)

Materials and Methods: During a 10 month period, from October 2006 to July 2007, 180 infants were hospitalised in a 20 bed NICU. Surveillance cultures of nasopharyngeal and anal swab specimens were performed regularly to every neonate admitted to the NICU, and every 2 weeks until their discharge. Twenty two infants were colonised by *A. baumannii*. The isolated strains of *A. baumannii* were identified and tested for antibiotic susceptibility by VITEK 2 automated system (Biomerieux, France). The results of sensitivity tests were evaluated according to CLSI recommendations. The isolates proved to be resistant to aminoglycosides, cephalosporins, penems, co-trimoxazole and ciprofloxacin and sensitive only to colimycin, doxycycline and tetracycline. Five of those neonates developed bacteraemia and one of them died from sepsis. Environmental surveillance did not reveal the *A. baumannii* source. Epidemiological investigation revealed that acinetobacter entered the NICU in October with a colonised neonate coming from a maternity Hospital in a distant area where the microorganism was abundant. The infection control committee of the hospital reinforced the usual preventive measures as cohorting of the the colonised/infected neonates and especially the proper hand hygiene protocol among healthcare workers in order to control the outbreak. *A. baumannii* disappeared from NICU after the discharge of the last colonised neonate.

Conclusions: Outbreaks of multi resistant bacteria is a major problem in NICU because of the prematurity of neonates, the consumption of antibiotics, the use of invasive devises and the prolonged hospitalisation. Surveillance cultures, followed by a good medical practice and standard hygienic precautions should be stressed out.

P797 *Burkholderia* spp. contamination detected in ultrasound gels

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Objectives: Contamination of pressure transducers, propofol, water used to dilute alcohol including skin antiseptics, mouthwash, antiseptics, etc has resulted in outbreaks of nosocomial infections. Similarly, there are also reports stating that contaminated ultrasound gels cause hospital infections. In this study, *Burkholderia* spp. contamination determined in ultrasound gels used in our hospital were investigated.

Methods: During surveillance studies performed for infection control in our hospital, an increase in wound infection rates were determined and environmental cultures were performed to determine the source of infection. In ultrasound gels, *Burkholderia* spp. was grown. Therefore, all gels in the hospital were collected to investigate for contamination. Gels were packed in boxes containing 12 plastic bottles, and inoculations into blood agar and EMB agar for culture were performed. Bacteria grown in cultures were defined by conventional methods and BBL Crystal Identification System (Becton Dickinson and Company). In order to investigate relationship with clonality, pulse-field gel electrophoresis was performed in identified origin.

Results: Two different brands of gels were found to be used in our hospital. A total of 669 unopened bottles of gels from two brands were sampled for cultures. When no growth was observed in any of total 364 bottles for one of the brands, these gels were distributed back to the hospital. As for the other brand, a total of 305 bottles were investigated and in 222 (72.8%) of them, similar colony growth was detected. One strain was taken per packet therefore total of 21 strains of *Burkholderia* spp., were defined. Pulse gel electrophoresis was performed for all 21 strains and was found that all originated from the same clone.

Conclusions: It should be considered that ultrasound gels may be contaminated during production and/or packaging and may be a source of nosocomial infections.

P798 Pseudo-outbreak of *Serratia marcescens* in a tertiary care hospital

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Objectives: To investigate an outbreak due to *Serratia marcescens* isolated from different samples (mostly cathether tips) of the patients.

Methods: Between September and November 2007, an apparent increase in positive culture results for *S. marcescens* was observed in the Clinical Microbiology Laboratory of Kocaeli University Hospital. A pseudo-outbreak was suspected and an outbreak investigation including retrospective and prospective studies using chart review, environmental sampling and random arbitrary polymorphic DNA-polymerase chain reaction (RAPD-PCR) of randomly selected isolates were performed by Infection Control Commitee.

Results: During the epidemic period, 67 strains of *S. marcescens* were isolated from the various specimens of both hospitalised and outpatients. Average isolation number was 4 strains/month of the bacteria in the previous months. Nine of 67 strains were belong to true infection. Thirty-five of them were isolated from cathether tips and 11 from deep tissue biopsy specimens. *S. marcescens* was also isolated from the saline solution in culturing area of the laboratory and recognised that he technician has been using the same stock saline solution for processing the specimens without changing needle. RAPD patterns of the isolates were identical with the pattern of saline strain. The contaminated saline solution was discarded, the technician was educated and no additional cases of suscepted contamination has been observed.

Conclusion: This pseudo-outbreak emphasise the importance of the periodic observation of specimen processing procedures and education of laboratory workers in the microbiology laboratory.

P799 A nosocomial outbreak of CTX-M producing *Serratia marcescens* in a Bulgarian neonatal hospital unit

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Objectives: The nosocomial spread of ESBL-producing *S. marcescens* remains a significant world-wide problem although in Bulgaria it have been rarely reported. The study investigates the epidemiology of a nosocomial outbreak of ESBL-producing *S. marcescens* in a neonatal intensive care unit.

Methods: A total of six infants were clinically studied. Three of them have experienced various clinical symptoms, one died with meningitis and two were clinically healthy. Six isolates *S. marcescens* were cultured from CSF (1), throat aspirate (3), nasal swabs (2). Identification, antimicrobial susceptibility and tests for ESBL production were performed by VITEK 2(BioMerieux, France) and conventional methods. PCR detection of CTX-M, SHV and TEM ESBL encoding genes was performed. Molecular typing was carried out by using ERIC-PCR and REP-PCR. The six isolates were compared with another set of 35 isolates originating from different hospitals from all over the country. The fragment analysis was carried out in a novel capillary electrophoresis instrument HDA-12 (eGene inc., USA) that generates results within eight minutes.

Results: The antibiotic susceptibility testing revealed two types. Five isolates were multidrug resistant, susceptible only to fluoroquinolones and carbapenems and one isolate was generally susceptible. Only CTX-M gene was found among the five MDR strains. The molecular typing grouped the isolates in two clusters – one containing the five identical MDR isolates and a second one including the unrelated susceptible strain. The two methods applied generated concordant results.

The general comparison with the other nosocomial isolates from the country revealed a total of five genotypes.
Conclusions: This study is the first to present molecular diversity of nosocomial *S. marcescens* isolates from Bulgaria. The applied PCR typing systems ERIC-PCR and REP-PCR proved efficient for discrimination of closely related as well as unrelated nosocomial isolates.

P800 Nosocomial outbreaks caused by *Serratia marcescens*

L. Lazarevska (Skopje, MK)

Background: Over the course of 2005, we registered and investigated two nosocomial outbreaks caused by *Serratia marcescens*. The first one occurred in the neonatal intensive care unit (NICU), at the Clinic of gynaecology and obstetrics, and the second one in the paediatric intensive care unit (ICU) of the Clinic for Children's Diseases – Medical Faculty – Skopje, R. Macedonia.
Methods: The initial and final Information were elaborated by the Department of Epidemiology and the Department of Microbiology at the PHO Institute for health protection-Skopje. During the outbreaks, throat, nose, and eye swabs, as well as blood samples were taken from every patient hospitalised in the ICUs. Materials from working surfaces and medical staff were taken for bacteriological investigation.
Results: *Case A*: In January 2005, 10 premature infants in NICU were infected (n = 5) or colonised (n = 5) by *Serratia marcescens*. Four of them developed septicaemia, which was fatal for one of the babies. One baby had conjunctivitis, and five babies had no clinical symptoms.
Case B: In July, 2005, seventeen newborn babies were registered in outbreaks caused by *Serratia marcescens* in ICU. Five babies suffered from septicaemia, in ten cases bacteria were isolated from the trachea, in one case from the nose and there was one case with eye-isolated bacteria.
Apart from *Serratia marcescens*, we registered five cases with *Klebsiella aerogenes* (n = 5) and *Pseudomonas aeruginosa* (n = 5).
Only six babies manifested clinical symptoms and all babies were treated with antibiotic regimen.
Conclusions: Throughout both nosocomial outbreaks, the units were partially closed, the infected newborn babies were isolated and a general cleaning was carried out, as well as disinfecting of all units. The supply of antibacterial soap was replenished and the babies were not breast-fed, but put on formula feeding.
A focal source for all micro-organisms was not identified, but we believe that bacteria transmitted via contaminated hands play an important role in the outbreaks. Cohorting, isolation of colonised and infected newborn babies, and rigorous environmental hygiene were crucial measures in preventing further hospital infections.

P801 Measles outbreak and serological testing of healthcare workers in a Spanish hospital

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Background: In 1981 the combined measles-mumps-rubella vaccine was introduced in Catalonia. In 2006 the Public Health Department reported that the prevalence of IgG measles antibodies was ~99% in children and adult population. Recently, a measles community outbreak occurred in our area. A patient was diagnosed of measles on his 7th day of hospital stay. CDC recommendations state that healthcare workers (HCW) with direct patient contact and without documented immunity should be vaccinated as soon as possible and relieved from direct patient contact, from the 5th to 21st day after exposure. This measure is costly and represents a considerable burden for the hospital. The objective of the study was to determine the prevalence of measles antibodies among HCW and to evaluate a questionnaire as a screening tool.
Material and Methods: This was an observational study in an acute care hospital in Terrassa, Spain. All HCWs born between 1966 and 1980 were included. A questionnaire regarding previous exposure or immunisation with the triple vaccine was used. ELISA immunoassay was used to measure measles IgG antibody titres. HCW with exposure to the index case were relieved from direct patient contact until final serological results were obtained.
Results: 44 HCW were exposed. Only one seronegative HCW was relieved from patient contact for 15 days. 586 (91.7%) out of 639 HCW were tested. 6 (1%) refused serological testing, 78.4% were women. Median age was 33 years. 74% were physicians and nurses. 22.5% referred a previous measles episode, 29% previous vaccination, 4.2% referred both, and 55% did not refer any previous disease or vaccination. The seroprevalence was 90%, 81%, 80%, and 86%, respectively. In the whole cohort the prevalence of IgG antibodies was 86%. The sensibility of the questionnaire was 85.6% and the specificity was 14%.
Conclusions: Seroprevalence of measles antibodies in HCWs was high but lower than expected. Data obtained through personal interview did not provide accurate information as to be used as a surrogate for immunity. In case of a nosocomial outbreak, the determination of the immune status in exposed HCW is useful in order to avoid unnecessary interventions. All HCWs must be tested and, if necessary, vaccinated before their incorporation into the hospital.

P802 Uncommon nosocomial transmission of TBC in an outpatient clinic in 2005: possible route of pathogen

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Objectives: We report an uncommon nosocomial transmission of *Mycobacterium tuberculosis* in an outpatient ear, nose and throat (ENT) clinic. The first patient suffered from an acute otitis media. The second patient complained about sudden deafness. The index patient was diagnosed with tuberculosis otitis media 3 months after the initial diagnosis. The second patient was diagnosed with tuberculosis otitis media 5 months after the initial diagnosis and 3 months after the index patient.
We investigated the contact situation regarding the two patients and the probable route of transmission.
Methods: The case histories were checked for possible contact between the two patients. The similarity of the pathogens was investigated by molecular methods. The hygienic situation of the outpatient clinic was audited and the hygienic plausibility of the transmission of the pathogens was investigated by laboratory experiments. Potential other contacts in the clinic were traced back and investigated using a t-cell based assay (Elispot) for IFN-gamma.
Results: Both patients met once in the ENT clinic. Both had an invasive ear examination on this day. Microbial investigation revealed the same clonal pattern.
The audit revealed incomplete compliance with hygienic necessities, especially concerning hand disinfection and environmental disinfection between different consultations (e.g. contact to ear microscope). In addition, staff reported that the rinse water of the ear suction device was not changed between consultations. An experiment proved a possible contamination of the suction device by contaminated rinse water. A second experiment showed that the flyback of rinse water in the upside down hanging suction device was able to contaminate the apex of the device.
None of the 10 contacts, who were treated in the ENT clinic on the same day revealed a positive result in the Elispot.
Conclusion: Investigations demonstrated probable transmission in the outpatient clinic. Different routes of transmission are possible. A lecture in hand disinfection was given and a new hygienic management was established which included systematic disinfection of patient environment between consultations. In addition structural changes like a hanger for the suction device were established. Hygienic management is a challenge in ENT clinics, as patient turnover is high, infectious disease are common and many medical devices are used.

P803 Audit of pulmonary tuberculosis management in a hospital with high tuberculosis prevalence: a tool for quality improvement in tuberculosis control

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Objectives: To avoid nosocomial TB transmission, infection-control measures, using administrative controls, engineering controls and personal respiratory protection are recommended by the CDC. An audit on the implementation of these administrative controls was performed at Centre Hospitalier Universitaire Saint Pierre (CHUSP), a public hospital in Brussels, Belgium, with high incidence of TB admissions. In countries with low TB incidence like Belgium, many doctors lack experience in recognition of RTB.

Methods: A retrospective observational study on all patients admitted to CHUSP, with suspicion of active respiratory TB (RTB) or with positive cultures for *M. tuberculosis* from a respiratory specimen, between January 1, 2002 and December 31, 2006. The use of airborne precautions (AP) was evaluated for each hospitalisation episode (HE). A decision tree for predicting active RTB based on 4 criteria (upper lung infiltrate at the chest X-Ray, fever, weight loss and CD4 count), elaborated by El-Solh and al. (Am J Respir Crit Care Med 1997; 155: 1711–16), was tested. Median TB management intervals (25th and 75th inter-quartiles) were calculated. Intervals exceeding 24 hours were considered to be a delay.

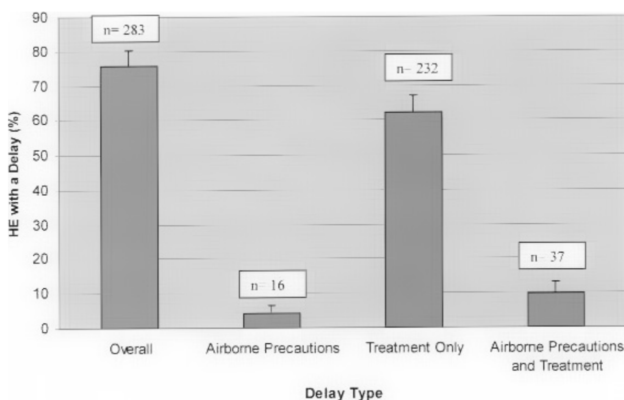

Figure: % of HE with delays in diagnosis and treatment in active RTB (n = 374).

Results: The incidence of active RTB from 2002 to 2006 was 4.25/1000 admissions. The cohort consisted of 971 HE. The ratio of active RTB to number of AP HE was 374/962. Among the 374 HE with active RTB, there were 92 AP failures (51 delays in TB suspicion, and 41 AP stopped prematurely). Theoretical delays in TB suspicion (10/364: 2.75%) generated with the decision tree were significantly inferior to the observed delays (46/364: 12.64%, p: 0.008). The median TB suspicion, treatment and overall management interval was: 0 (0–6) days, 3 (1–6) days and 4 days (1–7 days), respectively [figure: % of patients with delays in diagnosis and treatment in confirmed RTB (n = 374)].

Conclusions: APs are not correctly implemented in 25% of patients diagnosed with active RTB at CHUSP. Although overall TB management intervals seem reasonable, they are under-estimated, as some patients with active RTB are referred to CHUSP by other institutions. Results will be presented by distinguishing HE referred from other institutions to those HE entirely managed by CHUSP medical fellows. The retrospective utilisation of a decision tree has increased sensibility of RTB detection from 84% to 97%. Prospective evaluation of the tree is required to test specificity.

P804 The influence of patient heterogeneities on healthcare-associated infection, epidemic behaviour and control

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Objectives: Mathematical models have shown patient movement patterns to be important to healthcare-associated infection (HCAI) epidemic behaviour. Increased understanding of patient and hospital heterogeneities influencing these patterns would be of potential benefit for infection control. The objectives of this work are to: i) characterise the patient population and describe their movements within a healthcare network; ii) determine the consequences of these movements for HCAI epidemic behaviour and control.

Methods: We analysed seven years of admission data from a UK NHS Trust enabling us to fully characterise the demographics and network of movements between the three hospitals comprising the Trust. Consequences of these patterns for infection transmission and control were explored using mathematical models of infection spread, using both analytical and simulation-based methods.

Results: Despite most patients only visiting hospital a few times (40% being seen only once), for the final three years of data, readmitted patients constituted nearly 80% of admissions (numbers were lower in the first four years, attributable in part to left censoring), with approximately 80% of these being within one year.

Readmissions varied between demographic groups; most notably young women (21–30 yrs) and the elderly appeared to be ’core groups’ (the latter being a greater threat for HCAI). Analytical results showed effective isolation of these patients on admission (or clearing carriage at discharge) approximately halved their transmission potential.

Simulation results showed hospitals with the greatest number of admissions and whose patients were most likely to be readmitted had higher prevalences. Movement patterns were found to be critical to control success. For example: in a setting where each patient has, on average, 1.5 infectious admissions, admission screening has little benefit; whereas for an average of 2 infectious admissions, a strategy allocating 60% of its resources to screening the admission population is optimal.

Conclusions: Readmissions constitute a substantial proportion of the admission population and their consideration is likely to be crucial to the design of effective and cost-effective control strategies. Heterogeneities in movement patterns between hospitals imply optimal control strategies may vary between institutions. Future work will focus on investigation of tailored control strategies that identify and target patients at high risk of readmission.

P805 Scabies outbreak in an acute care setting involving 1,075 individuals

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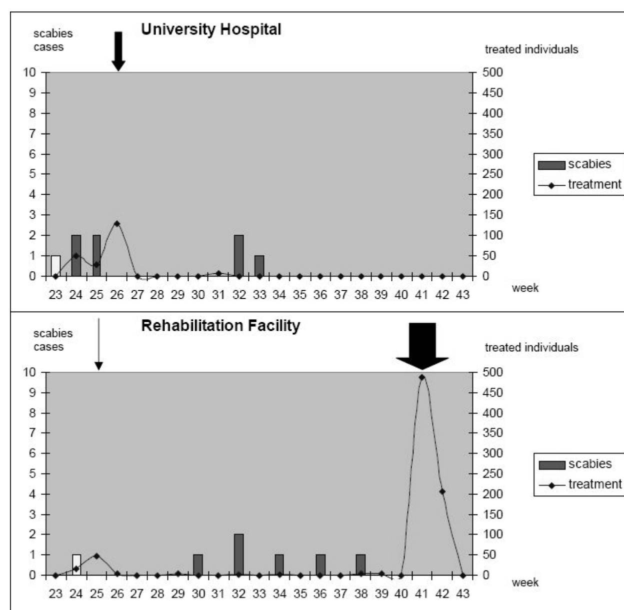
Objectives: Sarcoptes scabiei is an ectoparasite that causes either sporadical or in institutional outbreaks. We report a large outbreak in an University Hospital and a Rehabilitation Facility, that was controlled only after several months by strict infection control measures (IC).

Methods: *Setting:* University Hospital: primary and tertiary care centre with 750 beds, 39 intensive care unit (ICU) beds and 27,000 admissions per year. Rehabilitation Facility: primary and tertiary care centre with 92 beds (8 ICU beds) for patients with complex disabilities, spinal cord and brain injuries.

Epidemiological investigation: Scabies case definition: 1) positive skin scrape or 2) dermatoscopy or 3) typical burrows without history of prior scabicide treatment. Attack rate: The attack rate was defined as the number of symptomatic healthcare workers (HCW) in relation to all HCW exposed to scabies, and was evaluated by a standardised questionnaire.

Interventions: Individuals with scabies: treatment, contact precautions until 24 hours after initiation of treatment. HCW in contact with index case: preemptive treatment, including all household members (HM).

Patients in contact with the index case or infected HCW: dermatological examination, later preemptive treatment for all patients.
Results: In June 2007, a HIV patient was diagnosed with Norwegian scabies (crusted scabies) after a 6 week hospitalisation in the ICU of an University Hospital and consecutive transfer to a Rehabilitation Facility.
University Hospital: 4 HCW and 3 HM and no patients were diagnosed with scabies within 9 weeks from June to August 2007. The attack rate was 18% in HCW overall, 27% for HCW with direct contact to the index case and 10% for HM. The outbreak was finished in August after initiation of strict IC.
Rehabilitation Facility: No diagnosis of scabies was made at the beginning of the outbreak. Two HCW, 2 HM and 2 patients were diagnosed with scabies from July to September 2007 due to difficult implementation of IC. The attack rate of HCW was 3.9%, and 12.9% for HCW with direct contact to the index case. The outbreak stopped after simultaneous preemptive treatment of all HCW and patients in the Rehabilitation Facility.
Overall, 1075 individuals underwent preemptive treatment.
Conclusions: Case finding during scabies outbreaks is impaired by uncertain diagnostic tests in the early stage of disease. Therefore, in a hospital setting, early broad preemptive treatment and strict IC are essential for control.



Healthcare-associated infections

P806 Prevalence of nosocomial infections in Lithuania

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Objectives: The main objective of the surveys was to determine prevalence of nosocomial infections and risk factors in Lithuania.
Methods: Nation-wide point prevalence surveys of nosocomial infection (NI) were undertaken in 2003, 2005 and 2007 in 39, 35 and 30 acute hospitals correspondingly. Data were collected during bedside interviews by the medical doctors recording current status of patient. CDC criteria were used for NI diagnosis.
Results: There were app 8000 patients evaluated each year. The prevalence of nosocomial infections revealed was in 2003 – 4.3%, 2005 – 3.4%, 2007 – 3.5%. Prevalence of hospital acquired infections varied widely between departments wit top level in intensive care (app. 20%). It was clear tendency of increasing NI prevalence in nursing departments, where NI prevalence in 2007 (6.3%) exceeded surgical (4.6%) and trauma (5.5%) departments. The most prevalent NI were those of respiratory tract, accounting (accounting up to 30.1–44.3% of all NI) and surgical site infections (14.7–22.6%). Prevalence of risk factors was significantly higher in university hospitals compared to regional and local in all three surweys, but there was no difference in nosocomial infection prevalence rates.
Conclusion: Prevalence studies give the realistic view on NI problem, allows to follow national NI tendencies and gives valuable data needed for NI control.

P807 Increasing numbers of *Rhodococcus* infections at a large university hospital NHS Trust, Nottingham – An investigation

S. Capewell, J. Palfreyman (Nottingham, UK)

Objectives: Towards the end of October 2003 an unusual organism was grown from a number of paediatric blood culture bottles from one of the children on the Oncology Ward at the Nottingham City Hospital Campus. Routine Laboratory identification Tests as to the identification and significance of this Gram-positive organism were inconclusive. The Reference laboratory subsequently identified the organism as *Rhodococcus* sp. (not *Rh. equi*). The isolation of this type of organism increased over the following months and it was decided that an epidemiological investigation was required to find the source of infection and to prevent further outbreaks in a ward of vulnerable children.
Methods: A number of epidemiological methods were engaged to assess the outbreak. These included a study as to where the patient came from, a case assessment including time from line to infection, line usage, age and sex, as well as what type of underlying disease. A review as undertaken to see if this increase in infections caused by *Rhodococcus* sp. was occurring elsewhere in the hospital or the United Kingdom. Finally screening and environmental swabs were taken as well as a generally survey of the ward.
Results: There were no conclusions or similarities to be drawn from the case reviews or screening swabs. This increase in *Rhodococcus* infections had not been noticed in any other areas of the hospital or elsewhere within the region or country. On investigation of the Ward it was found that the HEPA filters had never been changed. These were removed and swabbed. *Rhodococcus* sp. was grown from 3 areas of the filters. Since their removal no more cases have been seen at Nottingham University Hospitals.
Conclusions: This was a nosocomial infection. The HEPA filters were the source of the *Rhodococcus* sp. infections seen in the Oncology ward at Nottingham University Hospital.

P808 Clinical and autopsy diagnosis of infectious diseases at an Italian teaching hospital

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Objectives: To evaluate the frequency of autopsy diagnosis of infectious diseases and to compare clinical and autopsy findings of infectious diseases at an Italian teaching hospital
Methods: A comparison of clinical and autopsy findings of cases studied between january 2001 and march 2007 at the Department of Pathology of Pisa hospital was made. A total number of 512 autopsies were performed in adult patients; 318 patients males and 194 females; mean age 65.0 yrs ±15.4 SD. 160 patients died in medical wards, 115 in intensive care units, 88 in surgical wards and 149 in emergency department. Particularly 201 cases with clinical diagnosis and/or autopsy diagnosis of infectious conditions were evaluated.
Results: 15 (7.5%) out of 201 autopsies showed no evident findings of infectious conditions while in the clinical diagnosis an infectious disease was included. 186 (36.3%) autopsies (114 males, 72 females) out of 512, revealed findings of infectious conditions involving 1 or more site of infection. Mean age of patients with autopsy findings of infectious disease was 63.3 yrs ±14.5 SD. In 72 (38.7%) out of 186 cases with autopsy findings of infections, no infectious diseases were diagnosed pre mortem. Underdiagnosis was most common in lung infections.
In 31 cases a clinical diagnosis of sepsis or septic shock was made. In 23 of these one or more sites of infection were evidentiated at autopsy. Infectious findings at autopsy included: pneumonia (n = 120), abdominal

infections (n = 27), meningitis or encephalitis (n = 8), pulmonar and/or extrapulmonary tubercolosis infections (n = 11), endocarditis (n = 15), invasive fungal infections (n = 12), AIDS/HIV (n = 3), HCV severe chronic hepatitis or cirrosis (n = 14), viral systemic infections caused by Herpesviridae (n = 8), urinary tract infections such as kidney abscess and acute pyelonephritis (n = 8).

Conclusions: These data underline the relative high frequency of infectious diseases at autopsy. The number of cases with clinically missed infectious conditions at autopsy was particularly high in lung infection and endocarditis. This fact confirms the difficulty in reaching a correct and timely diagnosis for this kind of infections in critical ill patients.

P809 Estimating disease burden of healthcare-associated infections in Finnish acute care hospitals

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Objectives: Few population-based estimates of disease burden for healthcare-associated infections (HAI) exist. We estimated the morbidity and mortality related to HAIs based on the data from the national prevalence survey performed in 2005. We also evaluated the completeness of the National Hospital Discharge Registry (HILMO) as a source of HAIs.

Methods: Data from the national prevalence survey constituted the basis for the register linkage. The survey included all inpatients (n = 3234) in acute care wards for adults in 30 hospitals. The Center for Disease Control and Prevention (CDC) definitions for HAIs were used and McCabe classification for comorbidity. In total, 753 HAIs were recorded in 703 patients (prevalence of HAI patients, 8.5%). Using the date of the prevalence survey and the patient's national identity code, data on hospitalisations including International Classification of Diseases (ICD)-10 codes for discharge diagnoses were obtained from HILMO, and the dates and causes of death from the National Population Information System. The incidence of HAI was calculated by Rhame and Sudderth formula using the prevalence (%), mean length of hospitalisations and mean number of days before HAI onset. Using the incidence of HAI and the 28-day case fatality of HAI patients in the prevalence survey, the annual estimates were extrapolated from the total number of hospitalisations in adult specialties in all Finnish acute care hospitals in 2005 (n = 304,456).

Results: In Finland (population, 5.2 million), we estimated that annually a total of 48,267 hospitalisations in adult acute care wards ended up to at least one HAI (9282 HAIs per million population) and 1514 patients with HAI died within 28 days (291 deaths per million population); 62% of the HAI patients who died did not have an ultimately fatal underlying disease (181 deaths per million population). In 33% of the hospitalisations with HAI, ICD-10 codes in HILMO indicated an infection. The proportions of HAIs coded in HILMO varied according to the severity of HAI: from 53% of surgical site/organ space infections, and 45% of pneumonias to 6% in urinary tract infections.

Conclusions: Our disease burden estimates can be used in healthcare planning and resource allocation. Notably, they did not cover paediatrics. The sensitivity of discharge diagnoses to recognise HAIs was low, but should not be overlooked as an additional case finding method, especially for severe HAIs.

P810 National 14-year survey of nosocomial meningitis in children and comparison of two periods, 1993–1998 to 1999–2006

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Introduction: In 1993–1998 and in 1999–2006 we have performed 2 surveys of paediatric bacterial meningitis in all 8 neurosurgery/paediatrics and infectious diseases departments in Slovakia.

Patients and Methods: In a prospective multicentric observational study, a prospective protocol was used to assess every case of meningitis appearing in clinically hospital stay in 0–18 years old patients in all 8 paediatric neonatal or neurosurgery departments in Slovakia. At least one positive CSF culture was involvement criteria. Mortality on infection, and/or CNS sequel and treatment (surgery, antibiotics) were analysed.

Results: 171 cases were enrolled. Comparing the two periods in our study of nosocomial meningitis 1994–1998 (A) to 1999–2004 (B) in aetiology, Gram-negative aetiology was more frequent in the second period (B) 40% vs. 2 5.3%, $p<0.05$). Vice versa fungi were more frequently obtained in the first period (8.7% vs. 2.0%, $p<0.05$). All *S. aureus* were oxacillin (OXA) resistant

Concerning Gram-negative bacteria *Acinetobacter baumannii* (14 vs. 6.1%) and *Pseudomonas aeruginosa* showed emergence (8 vs. 5.2%). Six (6) of 18 (30%) Enterobacteriaceae were cefepim resistant. Three *Ps. aeruginosa* isolates of 9 were resistant to meropenem.

Analysing mortality, there was no significant change in mortality among two periods of time (14.0% and 14.1%, NS), however there was a significant decrease of neurologic complications (e.g. hydrocephalus, palsy, epilepsy), in the 1999–2004 (29.9%) in comparison to 1993–1998, (42.9%, $p<0.01$). In contrast to that, cerebral abscess as a complication of meningitis appeared more frequently in 1994–2004 (14.3% vs. 3%, $p<0.02$).

Conclusion: There were no major changes in aetiology of nosocomial meningitis in children comparing 1993–1998 and 1999–2006, despite there was a trend towards in the increase of *Ps. aeruginosa* and *Acinetobacter baumannii* (9–12%), but the trend was only marginally significant for Gram-negative bacteria (35.5% vs 2 5.3%). Alarming was the level of resistance to teicoplanin in coagulase negative staphylococci – more than 10%. Mortality remains unchanged (12% in 1999–2006 in comparison to 15% in 1993–1998), but the number of neurologic sequellae decreased, probably because the proportion of inappropriate treated cases decreased from 20% to 10.3%. Nosocomial postoperative meningitis is still not a rare disease with 10–15% mortality and should be effectively prevented, and if occurs, radically treated with ATB.

P811 Risk factors and prognosis of external ventricular drainage-related infection

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Objective: To study the risk factors associated to external ventricular drainage (EVD) related infection.

Methods: Cohort study carried out since May 2004 to December 2006 in six tertiary hospital of Spanish Network for the Research in Infectious Diseases (REIPI). Inclusion criteria: Adult patients ($\geqslant$14 years) with EVD, during more than 24 h. Exclusion criteria: previous infection of central nervous system (CNS) at catheter's insertion day or death in the first 24 hours. All patients were followed until catheter withdrawal, infection, or exitus. Cases with EVD-related infection (EVDRI) defined by: a) clinical features (fever, meningeal signs, low Glasgow coma scale [< 14 or lower than previous status]); b) CSF findings (pleocytosis $\geqslant$100/UL, hipoglycorrachia < 40 mg/dL or CSF/blood glucose ratio <40%, and increased protein level > 100 mg/dL); and c) microbiological criteria (positive CSF Gram-stain, positive culture of CSF and/or catheter's tip). Patients without EVDRI were compared with cases. The end-points were the EVDRI and exitus, and the independent variables were: sex, age, diagnosis at admission, underlying diseases, prophylaxis, concomitant infections, previous antibiotic treatment, and those related to catheter use, including the need of catheter exchange. To control the interaction/confusion between variables a model of binary logistic regression adjusted by Wald test was used; significant differences were defined as $p< 0.05$.

Results: 323 patients were included, the median of catheterisation time was 10 days (2–88) days. The infection rate was 11.4% (37 patients). Thirty-four (91.8%) were monomicrobial; aetiological agents: Gram-positive cocci 17 (32.5%), Gram-negative rods 20 (50%), others 3 (7.5%). Independent risk factors associated to EVDRI were: age (OR 0.958, CI 95% 0.93–0.98); underlying disease (OR 2.80, CI 95% 1.11–7.07); Apache II (OR 1.12, CI 95% 1.04–1.22); catheter exchange (OR

9.58, CI 95% 3.14–29.20), prophylaxis at first catheter (OR 0.20, CI 95% 0.07–0.66). The mortality rate was 24.3% in EVDRI group and 21% non-infected patients (p = NS).
Conclusions: EVDRI is affected by the need of catheter exchange, the initial severity of patients, and comorbidity. Prophylaxis before the insertion reduced the EVDRI. EVDRI is not associated to higher mortality.

P812 Clinical significance of nosocomial spontaneous bacterial peritonitis in liver cirrhosis patients and impact of the 3rd generation cephalosporin resistance of Gram-negative bacilli on the outcome

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Background: There have been few reports of the aetiologies and treatment outcome for nosocomial spontaneous bacterial peritonitis (SBP) in liver cirrhosis (LC) patients.
Objectives and Methods: We performed a retrospective cohort study to compare the microbiological and clinical characteristics and outcome between nosocomial and community-acquired SBP in LC patients. All cases of SBP, in which culture was proven at Samsung Medical Center from Jan. 2000 to Jun. 2007 were included. Medical records and laboratory data were reviewed. Nosocomial SBP was defined as SBP diagnosed after 72 hours of hospitalisation.
Results: A total of 236 patients with SBP were enrolled (mean age, 56.6±10.7 years; male/female, 166/70). Nosocomial and community-acquired SBP were 126 and 110 cases, respectively. Among 239 microorganisms isolated, *Escherichia coli* accounted for 43.2%, *Klebsiella* spp. 14%, *Streptococcus* spp. 9.7%, *Enterococcus* spp. 8.1% and *Staphylococcus aureus* 5.1%. The overall mortality rate of nosocomial SBP was higher than that of community-acquired SBP (58.7% vs. 37.3%, p = 0.001). Nosocomial isolates of Gram-negative bacilli (GNB) were significantly more resistant to 3rd generation cephalosporin (42.1% vs. 10.0%, p < 0.001) and quinolone (30.9% vs. 50.0%, p = 0.003) than community isolates. Multivariate analysis revealed that nosocomial infection, concomitant hepatocellular carcinoma, presentation with shock, elevated creatinine and resistance to 3rd generation cephalosporin of GNB were significant risk factors for the 30-day mortality in SBP.
Conclusions: Nosocomial SBP has a poorer outcome than community-acquired SBP. The resistance to the 3rd generation cephalosporin of GNB, which is more frequent in nosocomial SBP than in community-acquired SBP, adversely affects the outcome of SBP in LC patients.

P813 Significance of positive urine cultures during the immediate post-trasnplant period in renal transplant recipients. A retrospective study

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Purpose: Urinary tract infections (UTI) are the commonest bacterial infections in renal transplant recipients. The study of UTIs in the immediate post-transplant period is of particular importance (higher degree of immunosuppression, presence of foreign bodies, exposure to nosocomial pathogens). Purpose of this retrospective study was to audit UTIs during the first post-transplant month and identify potential risk factors.
Patients and Methods: Renal transplant recipients with complete urine culture (UC) and urinalysis data for the first post-transplant month (period of September 2002-September 2007) were included in the study. All recipients, besides the induction therapy and triple immunosuppressive regimen, were treated with prophylactic antibiotics, namely intravenous cephotaxime for 10 days and then per os ciprofloxacin for six weeks.
Results: 116 renal transplant recipients (mean age 50.6±9.7 years, male gender in 74.1%) were studied. During the follow-up period of one month, 50 positive UCs were identified, derived from 38 (32.7%) recipients. A total of 27 recipients had only one positive UC. Urinalysis identified pyuria in 18 out of 38 recipients. Most frequent pathogens were Gram(+) bacteria (22/50.44%) (*Enterococcus faecalis* commonest), followed by Gram(–) bacteria (17/50.36%) (*Pseudomonas aeruginosa* commonest) and fungi (11/50, 22%) (3 *Candida albicans*, 8 *Candida* non-*albicans*). Mean time of identification of positive UC was 14±4.7 days post transplantation. Preliminary statistical analysis revealed significant association of probability of a positive UC with female gender (Pearson χ^2, p = 0.043) and cold ischaemia time (non-parametric test Mann Whitney U, p = 0.005). When the analysis included only the positive UCs accompanied with significant pyuria (defined as ⩾8–10 white cells/hpf), the above-mentioned associations were not reproduced.
Conclusions: Positive UCs were observed in 32.7% of renal transplant recipients, with *Enterococcus faecalis* and *Pseudomonas aeruginosa* being the commonest pathogens. It should be emphasised that all patients with positive UCs received specific antimicrobial therapy, irrespective of symptoms or puyria, due to concerns for defective inflammatory response. The issue of when to treat an isolated positive UC by a potential pathogen in the immediate post-transplant period has not been resolved. The probability of a positive UC in the first post-transplant month was related to female gender and cold ischaemia time.

P814 First results of ventilator-associated pneumonia results in five Dutch hospitals

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Objectives: Since 2005 Dutch hospitals can participate in the PREZIES surveillance of ventilator-associated pneumonia (VAP), in which the incidence of VAP as well of that of several risk factors are registered. These data are analysed to assess the most important risk factors in order to feedback case mix-adjusted data to the hospitals.
Methods: Data are collected and infections diagnosed based on the PREZIES-protocol, which is based on the CDC-definitions. Surveillance ended when a VAP was diagnosed or after a maximum of 28 ventilation days per period. Data were analysed with Cox regression.
Results: Five hospitals collected data on 457 ventilation periods (3098 ventilation days) with 411 patients. The average VAP incidence was 6.3% per ventilation period (9.4/1000 ventilation days). In univariate analysis neurosurgery compared to general surgery (HR 4.1), position in bed (supine compared to semirecumbent, HR 0.3) and SDD (HR 0.18) were significantly associated with the risk of VAP. Infection frequencies differed significantly per hospital. Therefore in multivariate analysis these data were adjusted for hospital and also for non significant risk factors with a p-value < 0.2 (age group).
In multivariate analysis the VAP risk was significantly higher for patients with a mean daily sedation score of four or higher (HR 6.4, 95% CI 2.6–15.7) and for patients on oropharyngeal prophylaxis (HR 16.7, CI 2.3–118). The risk for patients who were treated with multiple dose inhalers was lower, compared to those patients not treated with inspiratory medication (HR 0.36, 0.15–0.91). The interpretation of the results is hampered by the frequent presence of collinearity.
Most frequent organisms were *Klebsiella pneumoniae* (24%), *Pseudomonas aeruginosa* (13%), *Haemophilus influenzae* (11%) and *Staphylococcus aureus* (11%). In 9 of 29 cases two micro-organisms were cultured.
Conclusion: Oropharyngeal prophylaxis, the use of multiple dose inhalers and the average daily sedation score were significantly associated with the VAP incidence.

P815 Frequency and predictors of ventilator-associated pneumonia recurrence: a meta-analysis

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Objective: Large clinical series focusing on the risk factors associated with recurrence after the onset of an initial episode of ventilator-

associated pneumonia (VAP) produced inconsistent results. A meta-analysis would be helpful to shed light on the issue. To estimate the frequency of VAP recurrence and to identify risk factors associated with it.

Methods: PubMed, Scopus, Current Contents and references of retrieved articles were searched, without language restrictions. Pooled odds ratios (OR) and 95% confidence intervals (CI) were calculated by using both the Mantel–Haenszel fixed-effect and the DerSimonian-Laird random effects models.

Results: The overall frequency of VAP recurrence in 1,166 patients of the nine eligible reports was 23.7%. Among the twenty evaluated risk factors, only inappropriate empirical treatment (OR=1.99; 95% CI 1.06 – 3.72), acute lung injury/acute respiratory distress syndrome (OR=1.76; 95% CI 1.12–2.75) and shock (OR=1.55; 95% CI 1.01–2.41) at the day of diagnosis of the first VAP episode were found to be associated with VAP recurrence. There was also evidence, albeit inconsistent, that severity of illness at ICU admission was associated with VAP recurrence.

Conclusion: Recurrence involves almost one in four cases of VAP and is associated with inappropriate empirical treatment, acute lung injury/acute respiratory distress syndrome and shock, but not with first-episode causative pathogens. Recognition of these predictors may permit the timely implementation of measures to prevent recurrence of VAP.

P816 Closed tracheal suction systems for prevention of ventilator-associated pneumonia

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Objective: By meta-analysis of randomised controlled trials (RCTs), we assessed the evidence that closed tracheal suction systems (TSS) prevent ventilator-associated pneumonia (VAP).

Methods: We searched PubMed and Cochrane databases to identify RCTs that compared closed with open TSS for the management of mechanically ventilated (MV) patients.

Results: Nine RCTs were included in the meta-analysis. There was no difference on VAP incidence between patients managed with closed and open TSS [odds ratio (OR) = 0.96, 95% confidence intervals (CI) 0.72–1.28]. There was no heterogeneity among the eligible trials (I2= 0, 95% CI 0–0.65). The compared groups did not differ on mortality (OR= 1.04, 95% CI 0.78–1.39) and on intensive care unit (ICU) length of stay (one RCT: 12.3 ± 1.1 vs 11.5 ± 1.4 days, and the other RCT: 15.6 ± 13.4 vs 19.9 ± 16.7 days). Suctioning with closed systems was associated with longer MV duration (weighted mean differences: 0.65 days, 95% CI, 0.28–1.03) and higher colonisation of the respiratory tract (OR= 2.88, 95% CI 1.50–5.52) than open TSS.

Discussion: The available evidence suggests that closed as opposed to open TSS usage did not provide any benefit on VAP incidence, mortality, or ICU stay of MV patients.

P817 Opinions of intensivists and nurses towards use of selective decontamination of the digestive tract and selective oropharyngeal decontamination

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In the past decades, use of Selective Decontamination of the Digestive tract (SDD) and Selective Oropharyngeal Decontamination (SOD) has been object of discussion. From May 2004 to July 2006, a large group-randomised, controlled cross-over multi-centre study (13 hospitals) was performed in the Netherlands, in which the effects of SDD and SOD as compared to standard care, were determined on patient survival and antibiotic resistance. As part of this trial, assumptions by intensivists and nurses on effect of SDD and SOD, as compared to standard care, were studied, and the influence of these assumptions on compliance to the study protocol was determined.

Questionnaires were developed, based upon semi-structured interviews, to inventarise experiences of intensivists and nurses with SDD and SOD, and to inventarise assumed effect. Furthermore physicians were asked to appraise current and expected (after application of SDD) mortality rate in the included patient group. In the last week of each six month study phase, during 24 hours, all nurses and physicians received the questionnaire, which could be filled in anonymously.

Divided over three studyperiods, 1024 questionnaires were returned by nurses and 253 by physicians. Expectations on effect of SDD increased as the study proceeded, both in nurses and physicians. Physicians expected less reduction in mortality when using SDD, as compared to SOD and standard care(significant difference). The assumed relative reduction in mortality as appraised by physicians raised as the study proceeded.

Application of SDD was considered time consuming and annoying for patients, but increasingly effective.

P818 Risk factor analysis of deep sternal wound infections after cardiac surgery

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Objective: Deep sternal wound infections (DSWI) represent a severe problem in cardiac surgery. We observed an increasing incidence of DSWI in our university hospital in the period from January 2006 to September 2007. Therefore a case control study was performed to identify significant risk factors.

Methods: All cases of DSWI were recorded using CDC definitions over a 21 months period. All patients directly operated following a case patient were considered as control patients as long as they did not develop a surgical site infection. The following risk factors were recorded for each case and control patient: diabetes mellitus, BMI >25, COLD, renal insufficiency, nicotine abuse, immune suppression, preoperative exposure time, ASA-score, risk class stratification; date and duration of operation, time at extra corporal circulation, antibiotic prophylaxis, preoperative, intraoperative and postoperative serum blood glucose level, duration of mechanical ventilation, length of stay on ICU and in hospital. Univariate and multivariate analyses were performed.

Results: During the study period a total of 120 adult patients (72=male, 48= female) who underwent coronary artery bypass grafting (n= 91) or other cardiac surgery (n = 39) developed DSWI. On average the DSWI symptoms revealed 25 days after operation. Isolates cultured most often showed *S. epidermidis* (25%), followed by MRSA (19%), enterococci (14%), *S. aureus* (12%), coagulase-negative staphylococci (10%) and others (20%). The multivariable logistic regression model identified age greater than the median age of 68 years (OR 2.19; CI95 1.20–4.06), diabetes mellitus (OR 5.36; CI95 1.35–5.36), nicotine abuse or immune suppression (OR 5.46; CI95 1.91–17.58) and intraoperative blood glucose > 8 mg/dl (OR 2.27; CI95 1.17–4.42) as significant risk factors for DSWI. Preoperative antibiotic prophylaxis (OR 0.31; CI95 0.13–0.70) and extubation on the day of operation (OR 0.25; CI95 0.11–0.55) had a protective effect.

Conclusions: Our data mainly confirm the results of previous risk factor analysis. These findings should assist clinicians in identifying patients who are at increased risk of DSWI, as well as in developing strategies to minimise modifiable risks.

P819 Impact of skin and skin structure infections on hospital length of stay, mortality and costs: a matched cohort analysis

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Introduction: Skin and skin structure infections (SSSIs) are among the most common nosocomial infections. Given the high prevalence of SSSI infections, the potential economic impact from a health system perspective is considerable. Little is published in the literature on the impact of SSSI infections using real-world data.

Objective: To estimate excess hospital length of stay (LOS), mortality and costs attributable to SSSI.

Methods: Retrospective matched cohort study using Thomson Med-STAT data from the United States from 2001 to 2005. Patients $\geqslant$18 years of age, hospitalised $\geqslant$2 days with a secondary diagnosis of SSSI were

identified using ICD-9 codes and were matched with a control cohort of non-SSSI hospitalised patients using principal diagnosis, age, gender, and admission time frame as criteria. Up to 4 matched controls were randomly selected for each eligible SSSI case. Outcomes variables were LOS, total hospitalisation charges, and mortality. Fixed effect models and conditional logistic regression were used to analyse outcomes.
Results: Among the 1,472,965 hospitalisations, 23,681 cases were identified with secondary diagnosis of SSSI and matched with 90,235 patients (control). Cellulitis/abscess was most common (66.6%) followed by surgical site infections (21.6%), osteomyelitis (8.3%) and diabetic foot infections (2.8%). Microbiologically evaluable patients accounted for 11.7% of cases. Mean and median unadjusted LOS and charges for SSSI cases were 11.4 and 8 days and $51,311 and $27,942 compared with 6.3 and 4 days and $29,883 and $16,919 for controls. Regression models on LOS and total charges to adjustment for potential confounding variables, including frequency and number of therapeutic and diagnostic procedures performed, showed that SSSI cases incurred an additional 3.81 hospital days and $14,671. Similarly, adjusted odds-ratio of mortality between cases and control was 1.31 (95% CI 1.21–1.42) indicating an increased mortality risk in hospitalised patients with SSSIs as secondary diagnoses.
Conclusion: SSSI is associated with significantly higher LOS, total charges and mortality. Given the high number of SSSI that occur annually, the burden on the health system is considerable. More effective means to prevent or to treat SSSI will most likely be cost-saving.

P820 Outcomes and costs associated with monomicrobial and polymicrobial pathogens in patients with skin and skin structure infections

K.S. Akhras, S. Lin, A. Quintana, H.T. Hatoum (Raritan, Chicago, US)

Introduction: Although skin and skin structure infections (SSSIs) are caused predominantly by Gram-positive pathogens, polymicrobial infections and multidrug-resistant (MDR) pathogens are becoming a growing concern. Little is published about outcomes associated with monomicrobial versus polymicrobial SSSI.
Objective: To compare length of hospital stay (LOS) and hospital charges associated with monomicrobial and polymicrobial infections in patients with SSSI.
Methods: This retrospective analysis used Thomson MedSTAT data from the United States from 2001 to 2005. Patients ⩾18 years of age hospitalised ⩾2 days with a primary or secondary diagnosis of SSSI were identified using ICD-9 codes. Microbiology data were used to classify patients into microbiologically evaluable (ME) and non-microbiologically evaluable (non-ME). ME patients with infections were further classified into Gram-positive, Gram-negative, monomicrobial, and polymicrobial infections. Outcomes variables were LOS, total days of IV antibiotics and total hospitalisation charges.
Results: Of the 1,472,965 hospitalisations, 44,743 cases were identified as SSSI of which 5403 (12.1%) were ME. The overall unadjusted mean and median LOS and total charges for SSSI cases were 9.4 and 6 days and $40,150 and $ 20,911. Overall mean days of IV antibiotics therapy was 8 days. The table reports results by pathogen type for ME SSSI cases.

Unadjusted LOS and total hospitalisation charges by pathogen type

	Monomicrobial*		Polymicrobial*
	Gram-positive N = 2889	Gram-negative N = 907	All polymicrobial N = 1607
LOS, mean (median)	9.3 (6)	9.9 (7)	14.9 (8)
Antibiotics therapy days, mean	8.2	8.3	11.0
Total charges, mean (median)*	$37,684 ($19,053)	$42,001 ($20,771)	$68,538 ($26,789)

*Not all patients had charge data available.

Conclusion: In this large database, SSSI type infections caused by polymicrobial pathogens were associated with longer LOS, longer treatment duration with IV antibiotics, and higher total charges compared with monomicrobial infections.

P821 The epidemiology of hospitalised cases of skin and soft tissue infection in Europe

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Objectives: Data on complicated skin and soft tissue (SSTI) infections are scarce and a comprehensive assessment of all segments of SSTIs is not available. Therefore, the purpose of this study was to provide a thorough overview and in-depth insight to the burden of SSTI (distinguishing between community- and nosocomially-acquired infections) in European countries based on a detailed analysis of available statistical data, surveys and publications.
Methods: A multi-step approach using country-specific hospital discharge and surveillance data and data derived from a literature review were chosen to analyse the burden of SSTI in France, Germany, Italy, Spain and the UK. Relevant publications were identified through MEDLINE search of the medical literature (2000–2007) using the terms abscess, cellulitis, wound infection, ulcer wounds, skin and soft tissue infections. Annual hospitalisations for infections (cellulitis, abscesses, cysts) and underlying diseases susceptible to SSTI (burns, diabetic complications, decubitus ulcers, trauma) were extracted from annual country-specific hospital discharge surveys. Incidence rates for surgical site infections were obtained from the 2005 Hospital in Europe Link for Infection Control through Surveillance (HELICS).
Results: The analysis resulted in an estimated number of 1.3 million hospitalised SSTIs in 2004 in the EU-5. Of these, more than 25% were nosocomially acquired skin infections, while the majority of SSTIs were community-acquired or acquired in long-term care facilities. The largest proportion of all hospitalised SSTIs were cellulitis amounting to 52.7% of all SSTI, followed by surgical site infections with 15.8%, diabetic skin infections amounting to 15.3%, decubitus ulcer infections to 12%, traumatic wound infections (burns, fractures, other open wounds) to 4.2%.
Conclusion: These findings highlight the burden of SSTIs and associated underlying conditions in Europe. The summarised overview on all segments of SSTIs provides a better understanding of the large number of SSTIs and their origin.

P822 Post-operative bacterial meningitis: a 2-year study in a neurosurgery department

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Introduction: Post-operative bacterial meningitis is defined by a positive CSF culture in patients with clinical and laboratory findings indicative of meningitis and is associated with a wide variety of conditions requiring neurosurgical management.
Material and Methods: 745 patients who were operated on due to craniocerebral injury (CCI) or non-traumatic pathology (Non CCI) in the Neurosurgery Department of "Red Cross" Hospital, Athens, in the period 2005–2006. Meningitis, was diagnosed in 18 patients (14 male, 4 female), mean age 49.06 years. The records of patients were Statistical analysed by the statistical package SPSS 13 for Windows.
Results: Post-operative bacterial meningitis was observed in 2.4% (18) of the patients. The incidence rates meningitis among patients with CCI and those without CCI were 4.31% and 2.07%, respectively. Among patients with CCI, mortality reached 20%, while death was the final outcome of post-operative meningitis in 30.77% of the patients with non-traumatic pathology.
The mean age of patients who recovered fully was 46 years and that of patients who eventually died was 56 years..Age forms an independent prognostic factor for the outcome of postoperative meningitis ($p<0.05$).
In decreasing order of frequency, the isolated pathogens were as follows: *Acinetobacter baumannii*, *Staphylococcus* spp., *Enterococcus*

spp., *Enterobacter* spp., *Pseudomonas aeruginosa*, *Alcaligenes faecalis*, *Corynebacterium*. Eleven patients developed meningitis during their stay in ICU. Of these, 5 eventually died (45.45%). Mortality among patients stay in the Neurosurgery department was zero. Patients stay in ICU as a result to bad clinical-physical status they presented the worst outcome to post operative meningitis ($p<0.05$).
Conclusions: (1) Age was shown to be a statistically significant determinant of survival among those who developed post-operative meningitis ($p<0.05$). (2) The final outcome is worse in patients staying longer in ICU ($p<0.05$), compared to patients managed in the neurosurgery department. (3) The post-operative day that a CSF culture becames positive was not related to the final outcome of the CNS infection. (4) The incidence of resistant microbes mainly Gram(–) is increased.

P823 Treatment of joint prosthesis infection according to current recommendations improves outcome

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Objective: Evaluation of recently recommended treatment modalities for prosthetic joint infection (PJI).
Methods: A retrospective cohort analysis of 68 patients with PJI of hip or knee treated between 1995 and 2004 was conducted at the University Hospital Bern (Bern, Switzerland).
Results: A two-stage exchange was the most frequently (75%) surgical strategy, followed by retention/debridement (17.6%), one-stage exchange (5.9%), and resection arthroplasty or suppressive antimicrobial treatment (1.5%). The chosen strategy was in 88% in agreement with the recommendations. Adherence was only 17% for retention/debridement and 0% for one-stage-exchange. Most (84%) of PJI were treated with an adequate or partially adequate antimicrobial regimen. Recurrence free survival was observed in 51.5% of PJI episodes after 24 months follow-up. The risk of failure was significantly higher in PJI with a surgical strategy other than recommended (Hazard ratio (HR) 2.34, 95% confidence interval (CI) 1.10–4.70, $p=0.01$), and in PJI with antibiotic treatment not corresponding with recommendations (HR 3.45, CI 1.50–7.60, $p=0.002$). Other risk factors associated with lack of healing were a high infection score at the time of diagnosis (HR 1.29, CI 1.10–1.40, $p<0.001$) and presence of a sinus tract (HR 2.35, CI 1.10–5.0, $p=0.02$).
Conclusions: Our study demonstrates the value of current treatment recommendations. Inappropriate choice of conservative surgical strategies (such as debridement and retention) and inadequate antibiotic treatment are associated with failure.

P824 Tolerability of prolonged linezolid-rifampicin combination in bone joint infection: a retrospective study

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Background: High clinical success rate has been reported in patients (pts) treated with prolonged linezolid therapy (PLT) for bone joint infection (BJI) but bone marrow toxicity, especially anaemia, occurred in up to 25% of pts. Recently, a 35% decrease in the serum linezolid concentration was observed after the administration of 600 mg of rifampin due to a hypothetic increase in intestinal secretion of linezolid.
Objective: To assess whether linezolid-rifampicin combination (LRC) therapy reduces bone marrow toxicity compared with linezolid monotherapy or other linezolid combinations in patients with BJI.
Methods: Medical charts for pts with BJI treated with linezolid for at least 4 weeks were reviewed. Frequency of bone marrow toxicity events was assessed in pts treated with LRC and in those treated with linezolid monotherapy or other linezolid combinations. Anaemia was defined as haemoglobin value < 9.0g/dl, leukopenia as a total leucocyte count $<4\times10^9$/L, and thrombocytopenia as a platelet count $<100\times10^9$/L.
Results: 94 pts of mean age 53.5 ±17 years (range 18–94) treated for BJI (devices infection = 37%, chronic osteomyelitis=33%) between 1999 and 2006 were eligible for the study. Comorbidities included diabetes mellitus (27.6%), alcoholism (9.6%) and chronic renal failure (6.4%). 43 pts (44.8%) were treated with LRC, 25 (26.6%) with linezolid monotherapy and, 28 (29.7%) with other linezolid combinations. Surgery was required in 75 pts (79.78%). Culture of intraoperative samples and bone biopsy (122 microorganisms) were positive in 91pts (96.8%) [(meticillin resistant *Staphylococcus aureus*: 38 (31%), coagulase negative staphylococci: 46 (37.7%), others Gram-positive bacteria: 21 (17.2%), Gram-negative bacilli: 12 (9.8%), anaerobes: 5 (4%)]. Polymicrobial infections were seen in 21 pts (22.34%). Demographic characteristics were comparable between pts treated with either LRC or other linezolid regimens. Anaemia was recorded in 5 of the 43 LRC pts (11.6%) and, in 22 of the 51 pts (43%) treated with other linezolid regimens ($p= 0.001$). The mean delay from onset of anaemia and initiation of linezolid therapy was 13.4 ±8.7 weeks in LRC pts and 9.5 ±4.3 weeks in the other pts ($p=0.001$). 21/27 (77.8%) had to stop linezolid therapy and 15 of them had to be transfused. No other haematological adverse events were recorded.
Conclusion: PLT for BJI resulted in less anaemia episodes when it was combined with rifampin than when it was used in either monotherapy or other combinations.

P825 Risk factors and surgical site infection in a tertiary care hospital in Greece

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Objectives: To estimate the risk factors for surgical site infection (SSI) in the surgical wards of a tertiary care hospital in Greece.
Materials and Methods: In two surgical wards of 33 beds each, a total of 41 patients with surgical infections were recorded during the period 1/7/07 to 30/11/07. We applied logistic regression models to examine the effect of the following risk factors on the type of infection: underlying diseases (cardiac failure, diabetes mellitus, renal failure, respiratory failure, neoplastic disease, connective tisue disease, malnutrition, obesity, liver insufficiency), smoking, alcohol consumption, medications (chemotherapy agents, corticosteroids, others), American Society of Anesthesiologists (ASA) score, type of anaesthesia, type of surgery, duration of surgical procedure, postoperative time in Intensive Care Unit (ICU) and time on ventilator.
Results: Among 41 patients with surgical infections, 26 were males (63.41%) and 15 were females (36.59%) while the mean age was 62.02 years. Totally, 22 had SSI (53.66%), 3 had pneumonia (7.32%), 3 had bacteraemia (7.32%), 2 had intraabdominal abcesses (4.88%), 3 had pneumonia plus intraabdominal abcesses (7.32%), 1 had bacteraemia plus SSI (2.44%), 2 had central catheter infection plus SSI (4.88%), 1 had SSI plus intraabdominal abcess (2.44%), 1 had pneumonia plus bacteraemia plus SSI (2.44%), 1 had pneumonia plus SSI plus central catheter infection (2.44%), 1 had bacteraemia plus SSI plus central catheter infection (2.44%), and 1 had pneumonia plus bacteraemia plus SSI plus central catheter infection (2.44%).
Using logistic regression analysis, we found that only postoperative time in ICU or time on ventilator can increase almost 10 fold the risk of acquiring any surgical infection other than SSI (Odds ratio:10.8, 95% Confidence Interval:2.01–58.10).
Conclusion: More than half of the patients in this study experienced a surgical site infection. Postoperative time on ICU and time on ventilator affected significantly the type of infection.

P826 Arthroscopic knee surgery: postoperative wound infections, antibiotic prophylaxis and preoperative hair removal. Data from AMBU-KISS

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Objectives: In 2002, a database was set up within the context of the project AMBU-KISS (surveillance of surgical site infections

in ambulatory surgery) to document post-operative wound infections occurring in ambulatory surgical centres located in hospitals and medical practices. Reference data are compiled on internal quality assurance and contribute to the control of surgical site infections (SSI).

Methods: A SSI is diagnosed according to CDC criteria either during post-operative surveillance by the surgeon himself or by another doctor or family doctor. The reference data are made available to the participants in anonymous form every six months. Furthermore, AMBU-KISS participants were interviewed about perioperative prophylaxis and preoperative hair removal.

Results From 10/2002 to 6/2007, we received results from 101 centres performing arthroscopic knee surgery. A total of 71 infections occurred after 73,165 surgical interventions. The mean incidence was 0.08, i.e. 8 infections occurred after 10,000 operations. 40 centres completed the questionnaire. 36/40 participants removed the hair of their patients, mostly with razors (32/36) and immediately prior to surgery (31/36). 24/40 centres did not give perioperative prophylaxis, whereas 16/40 administered antibiotics for selected patients. 13/16 preferred 1st or 2nd generation cephalosporins as single shot (12/16) prophylaxis and applied them before incision (14/16). However, 4 centres administered antibiotics for 3–6 days post surgery or the evening before surgery (2/16).

Conclusions: The AMBU-KISS protocol is suitable for assessing and defining the magnitude of surgical site infections in ambulatory surgery, in which setting they were shown to be very low. However, there is still room for improvement with respect to the implementation of infection control guidelines. Guidelines do not recommend routine hair removal. If shaving is necessary for technical reasons, clippers instead of razors are recommended. More frequent use of broad spectrum prophylaxis than applying a single shot is not only useless but costly and may give rise to bacterial resistance. Generally, there is no evidence to support the use of antibiotic prophylaxis in arthroscopic knee surgery. Therefore, it is imperative that antibiotic prophylaxis be restricted to high-risk patients all the more in an ambulatory setting with very low infection rates.

P827 First case series of post-operative ocular infection due to *Streptococcus dysgalactiae* ssp. *equisimilis* in humans

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Clinical infection due to *Streptococcus dysgalactiae* ssp. *equisimilis* (*S. equisimilis*), including primary bacteraemia, pneumonia, endocarditis, arthritis, and streptococcal toxic shock syndrome are increasing; however, there has hitherto been only one report of ocular infection. In the present report, infection due to *S. equisimilis* is described in three immunocompetent patients after ocular surgery and in one theatre nurse. The three patients had a history of uncomplicated small incision cataract surgery with posterior chamber intraocular lens implantation. They developed pain and reduced vision in the operated eye 48 hours after surgery. On examination the affected eyes revealed dense peripheral corneal infiltrates with exudates in the anterior chamber. Gram-stained smears of vitreous fluid samples revealed numerous Gram-positive cocci in pairs and short chains, culture of these samples grew beta-haemolytic streptococci provisionally identified as *Streptococcus dysgalactiae* ssp. *equisimilis* by biochemical methods. The identification was confirmed by PCR for a 16S rRNA sequence specific to the species *S. dysgalactiae* and for a sequence of the streptokinase precursor gene specific to human isolates of the subspecies *equisimilis*.

Intravitreal cefazolin and amikacin along with topical ofloxacin and tobramycin for a few days resulted in resolution of infection in the patients.

The organism was also isolated from a throat swab sample collected from a 35-year-old healthy nurse who had assisted at the three surgeries. The nurse did not give a history of systemic infection and had no signs of upper respiratory tract infection or other diseases.

This is the first case series of postoperative ocular infection due to *S. equisimilis*. The infection may have been transmitted during surgery or the healthy carrier (theatre nurse) may also have transmitted the infection. Epidemiology of this organism is probably undervaluated and antibiotic treatment without bacterial identification is often done. Bacterial culture and identification by PCR followed by antimicrobial sensitivity testing are recommended in order to better investigate the spread and pathogenic potential of *S. equisimilis* and to evaluate the risk of nosocomial infections due to asymptomatic carriers.

P828 The effect of 3rd generation cephalosporin use restriction on antimicrobial resistance and clinical outcomes among intensive care unit patients

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Objectives: The primary goal of this study was to evaluate the effect of 3rd-generation cephalosporin restriction policy on the changes of the antimicrobial usage among patients admitted to ICU. The secondary goal was to investigate the changes of meticillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) colonisation rate. The third aim was to analyse the differences of the isolation rate of MRSA, VRE, 3rd-generation cephalosporin resistant *Acinetobacter baumannii*, extended spectrum β-lactamase (ESBL) producing *Escherichia coli* and *Klebsiella pneumoniae* from clinical specimens between pre- and post-restriction period. The fourth aim was to estimate the impact on clinical outcomes of patients, including hospital day, infection-related mortality and medical expenses.

Methods: This study was conducted between February 2006 and January 2007: a cross-sectional study with intervention measures. All patients admitted to the intensive care unit were eligible for investigation. Third-generation cephalosporin use restriction program was implemented at March 20, 2006. We compared the differences of antibiotic usage, bacterial resistance and clinical outcomes of patients between 1 month before restriction and after 8 months.

Results: Seventy-four percent reduction in cephalosporin use was observed after 3rd-generation cephalosporin restriction. This was accompanied with increments of piperacillin-tazobactam (781.3%) and 4th-generation cephalosporin (417.1%) use. There was no significant difference in colonisation rate of MRSA and VRE. The isolation rates of MRSA, VRE, 3rd-generation cephalosporin resistant *A. baumannii*, ESBL-producing *E. coli* and *K. pneumoniae* from clinical specimens of sputum and urine did not show significant differenc, while those from blood and CSF were positively correlated with the usage of 3rd-generation cephalosporin. In clinical outcomes, the infection-related mortality and the length of hospotal stay were not different. However, medical expense was decreased significantly.

Conclusion: Despite the significant decrease of 3rd-generation cephalosporin consumption after restriction policy, the bacterial resistance patterns remained unchanged. In clinical outcomes, medical expense was decreased significantly. Restriction of 3rd-generation cephalosporin was demonstrated to be efficient in the view of decreased invasive infections and economics among patients of ICU.

P829 Benefit of a single preoperative dose of antibiotics in a Sub-Saharan district hospital: minimal input, massive impact

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Objective: To evaluate the impact of a single shot preoperative antimicrobial prophylaxis in reducing the rate of surgical site infections (SSI) in an African hospital with very limited resources we implemented standardised guidelines with one dose of Amoxicillin/Clavulanic Acid within 2 hours prior incision.

Methods: In this rural district hospital in southern Tanzania an average of 150 surgical interventions is performed in two operating theatres every month. Ventilation is achieved by a defective air condition and open windows. Household soap is used for scrubbing.

Instruments are reprocessed by either heat (150°C for 1 h) or steam (134°C for 5min, temperature sensitive devices at 121 °C for 20min); a chemo-indicator is in use with every sterilisation process.

In a four month period in 2004, patients admitted for clean or clean-contaminated interventions had been included as pre-intervention group. The perioperative management differed depending on the surgeon. Patients admitted in the same setting in 2005 were enrolled as intervention group. They received one dose of 2.2g Augmentin® as intravenous infusion within 2 hours before incision. The substance was targeted at the bacteriologic pattern detected in SSIs of the pre-intervention group.
Patients were assessed daily and 30 days postoperative; in case of SSIs they received free diagnostic workup and treatment. Infections were identified according to the CDC classification; samples were analysed by Gram stain and agar (CHROMagar Orientation and CHROMagar *S. aureus*) in the local laboratory and the University Hospital Basel using standard methods. Data were reviewed by a senior infectious diseases specialist with full chart review.
Results: In the pre-intervention group 527 patients qualified for routine antimicrobial prophylaxis that was administered in 88% after incision and did not cover the expected pathogens to a large extent. One hundred and fourteen patients (21.6%) developed an SSI with 60% of detected pathogens being resistant to the administered antibiotics.
After implementation of the guidelines, the incidence of surgical site infections significantly decreased from 21.6% to 4% (11/276).
Conclusions: The implementation of a single shot antimicrobial prophylaxis dramatically decreased the rate of surgical site infections in a hospital with very limited resources. Such guidelines – though developed for industrialised countries – are even more effective in non-industrialised countries.

P830 Peritoneal dialysis-associated peritonitis: clinical features and predictors of outcome

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Objectives: To identify epidemiological, clinical and microbiological factors affecting the outcome of patients with peritoneal dialysis (PD)-associated peritonitis
Materials and Methods: All of patients with PD-associated peritonitis, admitted to the University Hospital of Heraklion, Crete, Greece from 1990 to 2007 were identified and their charts were retrospectively studied.
Results: A total of 247 episodes of PD-associated peritonitis occurring in 82 patients have been evaluated. The median age of patients was 68 years (range 10–92); 51 (62%) were males. Seventy-four (90%) had an underlying condition such as cardiovascular disease 48 (59%), diabetes mellitus 26 (32%), heart failure 17 (21%), autoimmune disease 6 (7%) and cancer 2 (2%). There were 104 episodes (42%) of Gram-positive, 46 (19%) of Gram-negative, 13 (5%) of polymicrobial and 11 (4%) of fungal peritonitis, while in 73 episodes (30%) cultures were negative. Regarding outcome, patients were divided into 2 groups: those with curable and those with complicated episodes. Complicated episodes were characterised by death due to peritonitis, relapse or repeated episode, removal of catheter and need for temporary or permanent haemodialysis. There were 183 (74%) curable, while 64 episodes (26%) were considered complicated and included 11 (4.5%) deaths, 22 (9%) relapses, 13 (5%) repeated episodes and 18 (7%) catheter removals. Multivariate analysis revealed that presence of purulent exit-site infection ($P<0.001$), peritoneal dialysis effluent cell count >100/μl for more than 5 days ($P<0.001$), prior antibiotic use during the last 3 months ($P<0.05$) and low serum total protein level on admission ($P<0.05$) were independent predictors of complicated episodes.
Conclusions: Exit-site infection, number of days with the peritoneal dialysis effluent cells count >100/μl, prior antibiotic use and serum total protein level are potential predictors of outcome in PD-associated peritonitis and may distinguish high-risk patients. Further study is needed to determine whether an aggressive management of these high-risk patients could improve outcome.

P831 Infections in patients with head injury: a 6-year retrospective study

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Objectives: Infections in patients with traumatic brain injury (TBI) are associated with prolonged hospitalisation and adverse outcomes. The acknowledgement of the pre-disposing risk factors may help decrease the morbidity and mortality
We conducted a retrospective cohort study to determine the incidence, bacteriology and risk factors for development of infection after head injury
Methods: The records of patients >18 years old, admitted with head injury in Crete Univ Med Ctr between 1999 and 2005 were abstracted. Data were analysed with SPSS
Results: 877 events were analysed with a total of 338 surgeries. Burr hole was the most common procedure (28%). Males predominated (75.6%) and the median age was 40. Surgical site infections (SSI) developed in 11.2% of the patients that underwent surgery. Wound infection was the most common (53%). Meningitis/ventriculitis comprised 43% of the SSI. Coagulase negative staphylococci (CNS) predominated as pathogens
Ventilator Associated Pneumonia (VAP) in 7.4% of the entries and they were the most common infections other than SSI. Gram(–) bacilli predominated in VAP with *Acinetobacter* spp. being the most common
Risk factors for SSI surgery through sinus ($p<0.001$), CSF leak($p=0.01$), ICU stay ($p<0.001$), use of a dural substitute ($p=0.01$), drain placement and duration of drains, ICP placement ($p=0.01$) and concomitant infections ($p<0.001$). Development of meningitis was associated with surgery through sinus ($p<0.001$), ICU stay ($p<0.0001$), CSF leak ($p<0.05$), use of ventricular drains ($p<0.01$) and concomitant infections ($p<0.001$). SSI and meningitis were associated with prolonged hospitalisation but only meningitis with an increased mortality ($p<0.05$). In multivariate analysis surgery through sinus, ICU stay were associated with development of SSI and meningitis but only SSI with CSF leak
Conclusions: Infections are common in patients with head injury. Superficial and deep SSI are associated with prolonged hospitalisation and increased mortality. Some of the risk factors may be minimised by careful surgical technique and aseptic management of foreign devices. The type and sensitivities of the pathogens must be determined institution by institution for effective empirical treatment

Lower respiratory tract infection

P832 Human Rhinovirus: a role in adult LRTI?

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Objectives: Human Rhinovirus (HRV) is a picornavirus often responsible for the "common cold". It has been associated with exacerbations of asthma and chronic obstructive pulmonary disease in adults and with bronchiolitis and pneumonia in children. However, there is limited information on HRV-associated lower respiratory tract infection (LRTI) in adults without chronic lung disease, partly due to few laboratories offering routine HRV detection in adults. The aims of this study were, firstly, to describe the epidemiology of LRTI associated with HRV in hospitalised patients, particularly, clinical outcomes in terms of chest consolidation, duration of hospital admission and mortality. Secondly, to compare these measures among patients with coexisting bacterial LRTI (HRV+bacterial), to those with HRV infection only.
Methods: Data were collected on 27 adult patients with respiratory secretions which tested positive for HRV who had been admitted to two University hospitals in Northern Ireland, between 2003 and 2006 with a primary diagnosis of LRTI. HRV was detected from respiratory secretions by a reverse-transcriptase polymerase chain reaction (RT-PCR). A bacterial isolate was defined as a positive result from at least one of the following: sputum culture; blood culture; atypical pulmonary

pathogen serology, legionella or streptococcal urinary antigen. Duration of hospital admission was expressed as geometric mean and interquartile range (IQ).
Results: Results are summarised in the table. There was no detectable significant difference between the patients groups in terms of age ($p=0.81$), sex distribution ($p=0.25$), presence of chest consolidation ($p=0.71$), duration of hospital admission ($p=0.46$), and mortality ($p=0.38$).

	Number of patients (M, F)	Mean age (years)	Consolidation present	Duration of admission (days)	Hospital mortality
Total cohort	27 (12, 15)	51.5	7 (25.9%)	13.2 (8.0, 16.0)	5 (19%)
HRV + bacterial	10 (7, 3)	52.8	3 (30.0%)	15.5 (8.8, 45.3)	1 (10%)
HRV only	17 (8, 9)	50.7	4 (23.5%)	12.0 (6.9, 16.0)	4 (24%)

Conclusions: Patients with a LRTI who isolated HRV only, had similar clinical outcomes in terms of duration of hospital stay, chest consolidation and mortality compared with patients with a bacterial LRTI who co-isolated HRV. Although the dataset is small, these findings suggest that HRV may have a role in the aetiology of LRTI in hospitalised adults.

P833 Inhaled corticosteroids increase the risk of pneumonia? A further step in a population-based study

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Aim of the study: To identify if the inhaled treatment general and inhaled corticoids in particular are risk factor for community-acquired pneumonia (CAP).
Patients and Methods: This was a population-based case-control study with a target population of 859,033 inhabitants >14 years of age. All patients with confirmed radiographic CAP presented over 1-year were prospectively registered ($n=3336$). Each case of confirmed CAP was frequency matched to a control subject ($n=3326$). Then we selected all chronic bronchitis or asthma diagnosis (CAP patients= 473 and controls=235) in order to compare the inhaler treatments. Then we adjusted for illness severity considering the use of oxygen therapy.
Results: Multivariable analysis including health habits, household and work circumstances, clinical conditions and comorbidities, and oral treatments, confirmed IT as independent risk factor of CAP. Among chronic bronchitis or asthma patients, IT remained a significant risk factor of CAP with a dose-related relationship, particularly when steroids or plastic pear-spacers were used (see table).

Variable	Cases (n = 473)	Controls (n = 235)	OR	95% CI	P value	OR (adjusted per O_2)	95% CI
Age: mean (SD)	59.6 (20.0)	60.9 (20.7)	0.99	0.99–1.00	0.99	–	–
Sex (% women)	189 (40.0%)	103 (43.8%)	0.85	0.62–1.17	0.324	–	–
Inhalers	183 (38.7%)	69 (29.4%)	1.52	1.08–2.12	0.015	1.43	1.00–2.03
Inhaled steroids	13 (4.3%)	1 (0.6%)	7.44	0.96–57.39	0.05	1.70	1.09–2.68
Inhaled beta-agonists	88 (23.3%)	43 (20.6%)	1.17	0.78–1.768	0.451	1.08	0.70–1.64
Inhaled anticholinergic drugs	84 (22.5%)	21 (11.2%)	2.29	1.368–3.83	0.002	2.17	1.26–3.75
Puffs/day							
1–6	77 (16.5%)	38 (16.5%)	1.16	0.75–1.79	0.002	1.11	0.71–1.74
6–10	41 (8.8%)	17 (7.4%)	1.38	0.76–2.51		1.32	0.72–2.42
>10	58 (12.4%)	9 (3.9%)	3.68	1.78–7.64		3.31	1.52–7.23
Inhalers							
Without spacer device	129 (28.8%)	55 (24.3%)	1.39	0.96–2.02	0.013	1.27	0.87–1.87
With spacer device	54 (12.1%)	14 (6.2)	2.28	1.23–4.25		2.02	1.06–3.88

Conclusion: IT is a significant and independent risk factor of CAP in the general population and among patients with chronic bronchitis or asthma, particularly when medication contained steroids or plastic pear-spacers were used.

P834 Use of ACE inhibitors and risk of community-acquired pneumonia

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Objectives: The administration of angiotensin-converting enzyme (ACE) inhibitors has been suggested as an alternative strategy for lowering the risk of community-acquired pneumonia (CAP) in the elderly, especially in patients with neurological and cerebrovascular comorbidity.
Methods: We critically examined the relevant data from studies regarding the impact of ACE inhibitors in lowering the risk of CAP and/or mortality due to CAP in the elderly. We searched in PubMed, Cochrane database and references of initially retrieved articles, and in http://clinicaltrials.gov for ongoing trials.
Results: We identified 285 evaluable studies. Eleven studies met the inclusion criteria and were evaluated further, of which 6 were prospective studies, 2 randomised controlled trials, and 3 retrospective studies. All studies including patients of Asian origin demonstrated a statistical significance in favour of ACE inhibitors. This was not the case, though, for studies including patients of non-Asian origin.
Conclusion: The available data suggest that ACE inhibitors may contribute to the reduction of the risk of CAP. Nevertheless, the clinical data are scarce and mainly comprise of studies including patients of Asian origin. Since there seem to be differences regarding the genetic polymorphism of ACE among patients of different origin, future studies may need to incorporate relevant genetics data that may help clarify the role, if any, of ACE inhibitors in prevention of CAP.

P835 Diabetes, glycaemic control and risk of hospitalisation with pneumonia: a population-based case-control study

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Objective: To examine whether diabetes is a risk factor for hospitalisation with pneumonia and to assess the impact of HbA1c level on such risk.
Methods: In this population-based case-control study we identified patients with a first-time pneumonia-related hospitalisation between 1997 and 2005, using healthcare databases in Northern Denmark. For each case, ten sex- and age-matched population controls were selected from Denmark's Civil Registration System. We used conditional logistic regression to compute odds ratios (ORs) as a measure of relative risk (RR) for pneumonia-related hospitalisation among persons with and without diabetes, controlling for comorbidity, alcoholism-related conditions, pre-admission use of antibiotics and immunosuppressants, marital status, presence of small children in the household, and degree of urbanisation.
Results: The study included 34,239 patients with a pneumonia-related hospitalisation and 342,390 population controls. The adjusted RR for pneumonia-related hospitalisation among persons with diabetes was 1.26 (95% confidence interval (CI) 1.21–1.31) compared with nondiabetic individuals. The adjusted RR was 4.43 (95% CI 3.40–5.77) for patients with Type 1 diabetes and 1.23 (95% CI 1.19–1.28) for patients with Type 2 diabetes. Diabetes duration $\geqslant$10 years increased the risk of a pneumonia-related hospitalisation (adjusted RR 1.37; 95% CI 1.28–1.47). Compared with persons without diabetes, the adjusted RR was 1.22 (95% CI 1.14–1.30) for diabetic persons whose HbA1c level was <7%, and 1.60 (95% CI 1.44–1.76) for diabetic persons whose HbA1c level was $\geqslant$9.
Conclusions: Type 1 diabetes, and to some extent Type 2 diabetes, are risk factors for a pneumonia-related hospitalisation. Poor long-term glycemic control among patients with diabetes clearly increases the risk of hospitalisation with pneumonia.

P836 Specific clinical determinants of community-acquired Legionnaires' disease associated with the Paris or Lorraine endemic strains

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Objectives: Legionnaires' disease (LD) is primarily caused by *Legionella pneumophila* sporadic or endemic strains. The Paris and the Lorraine strains are the main endemic strains in France. The objective was to explore the clinical determinants of infection associated with these two strains in patients with community acquired LD.
Methods: A double nested case-control study based on data from the French national surveillance network of incident LD between 1998 and 2005 was performed. All notified patients with a community acquired LD based on X-chest ray of pneumonia and definite biological diagnosis, were included. Cases were patients infected with Paris or Lorraine strains, whereas controls were those infected with sporadic strains. Epidemiological and clinical factors associated with Paris or Lorraine strains were assessed by adjusted odd-ratios, according to multivariate logistic regression models.
Results: Study population included 883 persons for whom infection occurred with sporadic (n = 364), Paris (n = 37) or Lorraine (n = 32) strains. Compared to patients infected with sporadic strains, female gender (adjusted odd-ratios [OR], 2.47, 95% confidence interval [CI], 1.44 to 4.25), ageing (OR for one year increase in age, 1.03, 95% CI, 1.01 to 1.05), treatment with steroids (OR, 3.47, 95% CI, 1.84 to 6.52) and history of hemopathy or cancer (OR, 1.90, 95% CI, 1.00 to 3.59) were more frequent among those infected with Paris strains. Compared to patients infected with sporadic strains, smoking (OR, 2.09, 95% CI, 1.11 to 3.96), diabetes (OR, 2.35, 95% CI, 1.12 to 4.91) and the 2004–2005 period of LD notification (OR, 8.30, 95% CI, 1.12 to 60.60) were independently associated among those infected with Lorraine strains.
Conclusion: Several patient characteristics were associated with endemic strains of *Legionella pneumophila*. These determinants were different for patients infected with Paris or Lorraine strains. These results observed in France should be extended to other areas to corroborate the potential link between the host susceptibility and a peculiar *Legionella pneumophila* strain background.

P837 Molecular epidemiology of *Legionella pneumophila* in Comunidad Valencia (Spain)

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Objectives: The main objective is to investigate the epidemiology of *Legionella* strains in a Spanish region that includes an area where legionellosis has become almost endemic, with continuous bouts of sporadic cases and several outbreaks affecting tens even hundreds of people, by using molecular and phylogenetic methods. Given that the culture efficiency of this bacterium from respiratory samples is very low, a minor objective was to obtain sequences derived from infected sputum without culturing it.
Methods: We have amplified and sequenced six protein coding loci and 3 intergenic regions for which we developed primers anchored in flanking conserved genes. Sequences of these nine markers were obtained in a dataset including about 100 clinical and more than 150 environmental samples, some related and others not to the clinical isolates, collected along several years. Environmental samples were sequenced from pure cultures while a semi-nested PCR approach was used to directly amplify and sequence DNA from sputum samples. When available, cultured clinical samples were also analysed. The obtained nucleotide sequences were used to infer evolutionary, population genetics and epidemiological relationships among them.
Results and Conclusions: Comparisons among sequences derived from isolates from the same patient (cultured isolate and DNA from sputum) were 100% identical, so amplification and sequencing from respiratory samples without culturing is feasible and more efficient.
Occasionally, clinical isolates from the same outbreak were very similar but not completely identical which, given the evolutionary rates in this bacterium, might be explained having different origins despite a common epidemiological pattern. For those cases with clinical and presumed environmental source isolates, we consistently observed lack of congruence between samples from both origins, probably because the origin of the infection was not actually identified. Therefore, the molecular analysis could complement the epidemiological investigation in order to correctly identify the environmental sources of infections.
Although we have detected a large genetic diversity in both clinical and environmental samples from the Comunidad Valenciana, our results indicate the existence of a predominant clone among clinical isolates which is the main responsible for recurrent outbreaks in a restricted area where legionellosis has become almost endemic.

P838 Penicillins vs. trimethoprim-based regimens for acute bacterial exacerbations of chronic bronchitis: a meta-analysis of randomised controlled trials

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Objectives: Semi-synthetic penicillins (SSP) (amoxicillin, ampicillin, pivampicillin) and trimethoprim-based regimens [TMP, co-trimoxazole (TMP/SMX), TMP/sulfadiazine) have been used for the treatment of patients with acute bacterial exacerbations of chronic bronchitis (ABECB).
Methods: We searched MEDLINE, Current Contents, and the Cochrane Central Register of Controlled Trials to identify and extract data from relevant RCTs for a meta-analysis comparing the effectiveness and toxicity of penicillins with trimethoprim-based regimens.
Results: Out of 134 RCTs identified in the search, 5 RCTs, involving 287 patients, were included in the analysis. There was no difference between patients with ABECB treated with SSP compared to those treated with TMP, alone or in combination with a sulfonamide, regarding treatment success [intention to treat (ITT) patients: 262, odds ratio (OR)=1.68, 95% confidence intervals (CI) 0.91–3.09, clinically evaluable (CE) patients: 246, OR=1.59, 95% CI 0.79–3.20] or drug-related adverse events in general (186 patients, OR=0.37, 95% CI 0.11–1.24), diarrhoea, skin rashes, or withdrawals due to adverse events (179 patients, OR=0.27, 95% CI 0.07–1.03).
Conclusions: Based on limited evidence leading to wide confidence intervals of the estimated treatment effects, SSP and trimethoprim-based regimens seem to be equivalent in terms of effectiveness and toxicity for ABECB.

P839 Treatment of patients hospitalised with community-acquired pneumonia using moxifloxacin versus levofloxacin and ceftriaxone: comparing costs from the hospital and social insurance perspectives

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Objective: To estimate the costs to German hospitals and statutory health insurers of managing patients hospitalised with CAP and treated with moxifloxacin (MXF) or a combination of levofloxacin plus ceftriaxone.
Methods: To ascertain costs from a hospital perspective, drug costs were taken from the Rote Liste; costs for hospitalisation were taken from a recent publication that reported bottom-up costing for the components of CAP management in 22 German hospitals; and costs for investigations and procedures were obtained using the German hospital fee scale, used for private patients. To estimate costs from a social insurance perspective, diagnosis-related group (DRG) codes including hospitalisation, medication, diagnostic and therapeutic procedures were used. Data on individual patient resource use were taken from a prospective, randomised, double-blind study of adults with CAP requiring hospitalisation and parenteral antibiotic therapy. Patients received sequential treatment with IV/oral antibiotics (either MXF [N=368], or comparator [N=365]) and for 7–14 days.

Results: As the percentage of patients reporting clinical response (MXF, 80%; comparator, 84%) met criteria for clinical equivalence, cost-minimisation analysis was appropriate. From a hospital perspective, mean per-patient costs were €2,190 (95% CI: €1,954, €2,463) for the MXF group, and €2,619 (95% CI: €2,422, €2,832) for the comparator group, with a between-treatment difference of –€430 (95% CI: –€740, –€138). From an insurer perspective mean per patient costs were €2,683 (95% CI: €2,576, €2,798) for the MXF group, and €2,669 (95% CI: €2,581, €2,768) for the comparator group, with a between-treatment difference of –€14 (95% CI: –€127, €152). Net income to the hospital, defined as the difference between the insurer payment and hospital cost, was €493 per patient for the MXF group (95% CI: €304, €664) and €50 (95% CI: –€110, €201) for the comparator group.
Conclusions: From a hospital perspective, treatment with MXF was significantly less costly than treatment with the comparator, with no clinically significant difference in outcomes. There was no significant difference in cost from an insurer perspective. This analysis found that compared with comparator, MXF reduced drug cost, had no significant effect on other costs and therefore reduced the per-patient cost of managing CAP.

P840 Excess death attributable to influenza in the Czech Republic in 1986–2006

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Objectives: Annual influenza epidemics differ in duration and magnitude. Influenza infection is underestimated, being easily mistaken for one of acute respiratory infections (ARI) since showing similar clinical symptoms. The aims of this study are to find correlation between mortality and influenza morbidity and to model mortality in different weeks of the year outside the influenza epidemic.
Methods: Data on daily deaths from all causes and deaths from diseases of the circulatory system in Czech Republic were available for 1986–2006 (altogether 2 317 522 and 1 267 718 deaths reported, respectively). Data on the incidence of influenza and other ARI were taken from the surveillance programme. The weeks in which ARI morbidity exceeded the epidemic threshold and at the same time, circulation of influenza virus among the population was reported by the National Influenza Centre, were considered as influenza epidemic weeks. Analysis was based on the assumption that outside the epidemic periods, deaths are distributed according to the Poisson distribution with a linear trend depending on time and with periodic behaviour during the year. The morbidity rate is only expected to increase in the epidemic compared to non-epidemic period.
Results: When comparing the weekly morbidity from acute respiratory illnesses and weekly mortality for all causes of death, the peaks of these two parameters almost overlap. In the epidemic period (152 weeks) 28.3% of findings were above the unilateral 95% tolerance limit of the model, compared to the non-epidemic period (926 weeks) with only 4.0% of findings above this limit. The mean estimated excess of annual deaths from all causes was 1922 (18.66 per 100 000). The median of deviations of the estimated number of deaths from the actual number of deaths is negligible at 11.9 for the non-epidemic period, being equal to 129.8 for the epidemic period, $p<0.001$. Similar results were found for deaths from diseases of the circulatory system accounting for 54.7% of all deaths in the study period.
Conclusions: The presented results confirm clearly and unambiguously excess in death rates during the influenza epidemic periods, depending on the duration and magnitude of the epidemic. The mean annual excess rate for Czech Republic is 1.7% population, the major part of this rate being attributable to influenza. Vaccination against influenza proved both effective and cost-effective and therefore is to be recommended as the most important preventive measure.

P841 Whooping cough in Catalonia, Spain

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Objective: to estimate the incidence of pertussis among general population, and to identify individuals with pertussis among the contacts of children with confirmed pertussis.
Methods: From April 2003 to April 2004, 465 pertussis-suspect-cases were reported from voluntary healthcare centres all over Catalonia. Pertussis infection was confirmed by means of culture and/or polymerase chain reaction assay of the nasopharyngeal specimens. We recorded a detailed cough history at presentation, as well as we asked parents for details of their child's immunisations and verified any information they gave from the parent held record or medical notes. A descriptive analysis was carried out of the demographic characteristics, clinical history, complications, treatment, diagnostic methods, epidemiologic information and vaccination.
Results: During the study period, there were a total of 71 laboratory confirmed cases of B. pertussis infection. There were 30 males and 41 females. Most (n = 31; 43.7%) of the patients were less than 1 year of age. Seven percent (5) of patients were between 1–6 years, 22.5% (16) were between 7 and 14 years and 26.8% (n = 39) were older than 14 years of age. Forty one cases (60%) presented paroxysmal cough, 22 (32%) post-tussive vomiting, 18 (26%) inspiratory whoop and 17 (25%) apnoea. The vaccination status was not known for 24 (33.8%) patients. Of the total vaccine status documented cases, 31 (66%) patients were vaccinated. Fifty patients (70.4%) were considered the index or co-index cases, 47 of them (94%) were children under 15 years old. Pertussis was confirmed in 18.3% (13/71) of the contacts of 50 index cases. Among these contacts identified as a pertussis case, 61.5% (8/13) were older than 14 years of age. These contacts were father (6 cases), mother (6), brother (2), sister (2), grandmother (2), others (3).
Conclusion: In this sample of cases of whooping cough arising in the highly vaccinated population of Catalonia, the overwhelming majority of cases were infants less than 1 year of age and older adults. Healthcare professional should be in alert to identify new cases of pertussis. The early recognition of pertussis index cases by healthcare professionals, the treatment of the cases and prophylaxis of the contacts is fundamental to control an outbreak in the community.

P842 Pertussis surveillance in one geographic area of Catalonia, 2003–2007

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Objectives: In Catalonia pertussis suspected cases should be reported to the Epidemiological Surveillance Unit. In 2003 a pilot surveillance program was undertaken, suspected clinical cases should be laboratory confirmed. Means to confirm cases were offered to public health services, mainly urgency wards. This report summarises the epidemiological factors associated with pertussis confirmed cases in one geographic area of Catalonia (Vallès Occidental) with 836077 inhabitants. Incidence rates and trends are described.
Methods: Clinical symptoms to suspect pertussis included cough for two weeks, and at least one of the following symptoms: dyspnea, paroxysm coughing, inspiratory whoop or post-tussive vomiting. Confirmation of cases requires PCR testing of nasopharyngeal fluid or Bordetella pertussis (BP) culture or to be epidemiologically linked to a laboratory confirmed case. Samples are analysed on a central laboratory of Hospital of Vall Hebron in Barcelona. Epidemiological data and vaccination status had been collected individually from all cases. Correct vaccination status was evaluated according to the Catalonia vaccine schedule and the age of each case.
Results: During the 2003–2007 period 127 confirmed cases were reported. Higher incidence was in 2003 and 2007 (until November) with 6.4 cases/100000 inhabitants and 5.6 cases/100000 inhabitants, respectively. Cases were more frequent on children under one year (35%)

and among 10–14 years (19%) but 30–39 years old cases were 14%. Most cases have received the doses of BP vaccine recommended according the age (47%). Two cases died (1.6% lethality rate), both had one month of live. PCR were more sensitive when it was done at the first days of disease.

Conclusion: The predominance of paediatric cases can be a real incidence of cases, it may be to an increased awareness of pertussis by paediatric physicians or typical symptoms of BP infection in children; or it may be a combination of all these factors. The role of cases on vaccinated patients should be taken into account to evaluate the vaccination strategies.

P843 New insights in determining the aetiology of severe community-acquired pneumonia and its implications

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Objectives: Severe community-acquired pneumonia (SCAP) can be caused by different bacteria which show similar clinical presentations. *Legionella pneumophila* (LP) can be difficult to diagnose and its prevalence might be underestimated. The main goals of this work were:

1. To evaluate the aetiology of SCAP, the prevalence of the aetiologic agents and its relationship with the clinical manifestations and the risk factors.
2. To assess the use of a new PCR method specific for LP as an analysis of routine.

Methods: A total of 256 specimens (BAL, blood and urine) from 96 patients (66 with SCAP and 30 controls) admitted in the Intensive Care Unit (ICU) were analysed, from December 2004 to November 2007. Etiological agents were assessed simultaneously by culturing, by urinary antigen test (UAT) and by PCR for LP. Kendall's Tau-b correlation, Mann-Whitney and Kruskall-Wallis tests, and Fisher's Chi-square were performed, using as dependent variables SCAP patients or LP-positives by PCR. Thirty-one independent variables were analysed, twenty-three describing symptoms, seven for risk factors and one for the causative agent. The level of significance was set at P<0.05.

Results: The most prevalent pathogen was LP (45.5%), followed by *Streptococcus pneumoniae* (25.8%) and others (8.2%). In 20.5% of the patients no aetiologic agents were detected. Polymicrobial infections were found in 24.2% of the patients with SCAP. LP-positives obtained by PCR were significantly (P<0.001) higher than those obtained by UAT. Smoking (P<0.05) and respiratory diseases (P<0.01) were identified as risk factors. On the other hand, fever, cough, fatigue, dyspnea, sputum, tachypnea, pulmonary shunt and infiltrate, amongst others, were symptoms of SCAP (P<0.005). Significant differences between SCAP and control patients (P<0.05) in the ICU length of stay were only observed when the infection was polymicrobial. Although a significant correlation (P = 0.001) was found between SCAP and LP positives by PCR, no correlation was observed (P>0.05) between SCAP and LP positives by UAT.

Conclusions:

1. The significant symptoms and risk factors found in this study should be taken into account when diagnosing and selecting the initial antibiotic therapy.
2. Polymicrobial infections present a worse prognosis and more agressive clinical evolution.
3. Urinary antigen test is not sensitive enough to determine SCAP positives, while PCR is a better and more powerful tool.

P844 CAPNETZ: Data on clinical efficacy of antibiotic therapy and mortality of CAP in Germany

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Objectives: Because of the medical and economic impact of community-acquired pneumonia (CAP), the Federal Ministry for Education and Research (BMBF) has, since 2001, been financing a network on CAP (CAPNETZ). CAPNETZ was founded to gain new insights into pathogenesis, particularly on pathogen-host interactions, to improve actual care in this area in Germany.

Methods: CAPNETZ integrates components of health research as well as clinical and basic research (horizontal network) on one hand and supports cooperation among general practitioners, community hospitals and universities (vertical network). The network partners report each suspected case of pneumonia to the local clinical centre of CAPNETZ. In case of CAP confirmation all clinical and microbiological data are documented.

Results: By the end of 2006, a total of 5,965 patients had been included in CAPNETZ of whom 1807 were out-patients and 4,158 were in-patients. The mean mortality was 8.2%. A further 5.8% of the patients died within the subsequent 5 months of observation after the cure of CAP. In spite of optimal conditions, an evaluable sputum sample could only be obtained from about half the patients. Usually a throat swab or nasal rinse was obtained for virus or mycoplasma diagnosis and urine was provided for antigen diagnosis (legionella and pneumococci). Even in most cases with sputum no causal pathogen could be found. *S. pneumoniae* was the most important pathogen, followed by *H. influenzae* and *M. pneumoniae*. With a prevalence of ca. 3%, legionella were rare. Nevertheless, they were linked to marked excessive mortality, emphasising the importance of performing a urine antigen test if there is a clinical suspicion of legionella. With a prevalence of about 3%, infections with Enterobacteriaceae were rare and primarily occurred in the risk group of very old and comorbid patients and patients from nursing homes. The mortality of these patients was 8-fold higher than in patients from a domestic environment. For the older patients (> 65 years), survival was highly dependent on the adequate primary antibiotic therapy. For this group (CRB-65 > 2), moxifloxacin was superior to β-lactam antibiotics. For patients with less severe disease (CRB-65 0 and 1), antibiotics like ampicillin were as effective as broad spectrum antibiotics.

Conclusion: The CAPNETZ data confirm the importance of pneumococci in CAP. The more severe cases benefit notably from an antibiotic therapy with moxifloxacin.

P845 The performance of CURB-65 in community-acquired pneumonia deteriorates with increasing age

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Background: CURB-65 has been validated as a predictor of mortality in community-acquired pneumonia (CAP) (Lim et al. Thorax 2003) and is recommended by the British Thoracic Society (BTS) and Infectious Diseases Society of America (IDSA) CAP guidelines to guide management decisions. There has been concern about the performance of CURB-65, however, in older adults (Myint et al, Age and Ageing 2006).

Objectives: To assess and compare the performance of CURB-65 in different age groups.

Method: Retrospective analyses of data prospectively collected for a CAP quality improvement study were performed (Barlow et al. Thorax 2007). Patients were stratified into four age-based cohorts: 16–64years (Group 1), 65–74years (Group 2), 75–84years (Group 3), and >85years (Group 4). For each age cohort, 30-day mortality was stratified by CURB-65 score. Sensitivity, specificity, positive predictive value, negative predictive value, and area under the receiver operator curve (AUROC) were then calculated. Statistical analyses were performed by SPSS (version 12) with a p-value of <0.05 taken to indicate statistical significance.

Results: 428 patients were included in the study (Group 1 = 132, Group 2 = 92, Group 3 = 128, Group 4 = 76); 53% female. Across the groups, there were significant differences in social circumstances, oximetry <92%, urea >7 mmol/l, new confusion, chronic illness, ITU admission and mortality. In patients with non-severe CAP (defined as CURB-65 = 0 to 2), 30-day mortality was 3% in Group 1 compared to 6%, 13%, and 27% in Groups 2 to 4, respectively. At the current cut-off of >3, sensitivity and specificity were 0.33 and 0.98 in Group

1, respectively, 0.77 and 0.62 in Group 2, 0.79 and 0.5 in Group 3, and 0.65 and 0.48 in Group 4. In Group 1, sensitivity and specificity were highest at a cut-off of >2 (1.0 and 0.83, respectively). AUROC was 0.93 (95% CI 0.88 to 0.99, p<0.001) in Group 1 versus 0.74 (0.58 to 0.90, p = 0.005) in Group 2, 0.74 (0.64 to 0.83, p<0.001) in Group 3, and 0.59 (0.46 to 0.73, p = 0.2) in Group 4.
Conclusion: Misclassification as non-severe CAP (CURB-65 = 0 to 2) of patients who subsequently died increased across the age cohorts. The performance characteristics of CURB-65 deteriorated with increasing age. Clinicians should interpret CURB-65 scores in elderly patients with caution, especially in those >75years. In those <65years, a score >2 may be a better indicator of severe CAP.

P846 The time to first antibiotic dose does not predict early clinical failure in severe community-acquired pneumonia

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Objectives: The time to first antibiotic dose (TFAD) has been raised as an important performance indicator in patients (pts) suspected of community-acquired pneumonia (CAP). However, the advice to minimise TFAD to 4 hours (4 h) is only based on retrospective database studies. Moreover, adopting TFAD as performance indicator may lead to prioritisation of pneumonia pts to others and unnecessary treatment for pts who ultimately receive a differential diagnosis, contributing to an increase in antibiotic (AB) use, resistance and costs. Therefore, we studied the effect of minimising TFAD on early clinical failure in a prospective setting.
Methods: Pts admitted because of severe CAP without previous AB treatment to our university hospital, were prospectively followed. On admission, pts' medical data and TFAD were recorded. Appropriate AB therapy was defined as all identified pathogens being susceptible for the initiated antibiotics. Early clinical failure was defined as a respiratory rate >25/min, peripheral oxygen saturation <90%, partial pressure of arterial oxygen <60 mmHg, haemodynamic instability or altered mental status on day 3 of hospitalisation. Multivariable assessment of factors predictive for early clinical failure was performed.
Results: 166 pts (mean PSI 108.8±24.1; age 67.2±14.5 year) were included. Overall, median TFAD was 3 h12min. In 100 pts (60.2%) TFAD was <4 h (median 2 h23min) and in 66 pts (39.8%) >4 h (median 5 h17min). No other significant differences were observed between both groups. In 82 pts (49.4%) aetiology could be established. Of these, 68 pts (84.1%) received appropriate AB therapy. 24 pts (26.4%) with TFAD <4 h had early clinical failure, as compared to 22 pts (35.5%) with TFAD >4 h (Odds Ratio (OR) 0.7; 95% CI 0.3–1.3; p= 0.22). In addition, after correction for pneumonia severity and appropriateness of AB therapy, no favorable effect of TFAD <4 h was found (p = 0.30). Furthermore, no time-effect relation could be identified in TFAD and early clinical failure (corr. 0.14; p>0.05). In multivariate analysis not TFAD but an arterial pH <7.35 on admission (OR 5.7; 95% CI 1.3–25.3), confusion on admission (OR 3.4; 95% CI 1.5–7.6) and *Staphylococcus aureus* infection (OR 7.2; 95% CI 1.3–39.6) were independently associated with early clinical failure (P<0.03).
Conclusions: Clinical parameters on admission other than TFAD predict early clinical failure in severe CAP. Therefore, implementation of TFAD as performance indicator may not be desirable.

P847 Evaluation of a new immunochromatographic assay for the detection of *Legionella* antigen in non-concentrated and concentrated urine samples

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Objectives: The aim of the present study was to evaluate the sensitivity and specificity of a new ICT: Uni-GoldTM *Legionella* Urinary Antigen (Trinity Biotech, Ireland) against the Binax Now *Legionella* test in non concentrated (NCU) and concentrated (CU) urine samples.
Methods: The samples tested were grouped into 3 categories;
Group 1: 80 NCU and CU samples from patients with pneumonia caused by *L. pneumophila*. Legionnaire's disease was diagnosed by isolating *L. pneumophila* from respiratory specimens and/or by serology and/or by urinary antigen detection (Bartels EIA).
Group 2: 38 NCU and 26 CU samples from patients with pneumonia of other aetiologies and also 22 NCU and 19 CU samples from patients with bacteraemia from a non-respiratory focus.
Group 3: 50 NCU and 20 CU samples from patients with urinary tract infections, with no signs of pneumonia.
Results: The Uni-GoldTM LUA test detected *Legionella* Urinary Antigen in 51/80 NCU samples from Group 1 patients (63.75% sensitivity). Binax Now test detected *Legionella* in 54/80 NCU samples from group 1 patients (67.50% sensitivity). Both tests were all negative for urine samples from Group 2 and 3 patients (100% of specificity). The overall concordance of the tests for NCU was 89.42% (k 0.961, SE: 0.023). (p: 0.083).
After concentration of the urine samples, Uni-GoldTM detected 76/80 (95% sensitivity) and Binax Now 78/80 (97.5% sensitivity) of the Group 1 samples. The specificity was 95.4% for Uni-GoldTM LUA and 100% for Binax Now. The overall concordance of the tests for CU was 96.5% (k 0.931, SE: 0.031). (p: 0.655).
Conclusions: UniGold test is a technique simple to perform, and the results are obtained in 15 minutes. Unigold when is compared to Binax Now doesn't show statistically significant differences. The concentration of antigen present in urine by ultrafiltration increased the sensitivity.

P848 Long-term course of clarithromycin for the treatment of stage II COPD patients with frequent exacerbations

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It was shown that standard courses of antibiotic treatment of exacerbation of chronic obstructive pulmonary disease (COPD) with macrolides or β-lactams have clinical benefits and may also reduce the frequency of further exacerbations. But the data about the long-term courses of macrolide treatment are still controversial.
The aim of the study was evaluate the safety and efficacy of long-term course of clarithromycin in comparison with standard course of amoxicillin/clavulanate potassium for the treatment of stage II COPD patients with frequent exacerbations.
Methods: 51 patients (32 male and 19 female) with exacerbation of stage II COPD were enrolled in randomised parallel-group multicentre trial to compare the efficacy of course of clarithromycin (CLA)(Fromilid, KRKA, Slovenia)with standard course of amoxicillin/clavulanate potassium (AMC) tablets (Amoksiklav, Lek d.d., Slovenia). All patients (mean age 57.3±1.1 years, smoking history 34.1±1.6 pack/years, FEV1 50.2±1.8 of predicted) had frequent exacerbations (3 or more per year)of COPD. *Chlamydophila pneumoniae* IgG antibodies in diagnostic titers were found in 70.5% patients. After randomisation 25 patients of AMC group received 625 mg of AMC three times a day during 10 days. 26 patients of CLA group were treated with 500 mg of CLA twice daily during 10 days then 250 mg once daily during 50 days. The follow-up period was 12 months. The study was conducted according to the GCP rules, Helsinki Declaration and Russian laws.
Results: No one patient has dropped out of the study. In CLA group there were 6 mild and 2 moderate, in AMC group – 2 mild and 5 moderate adverse events that did not need to stop the treatment. Dyspnea (MRC scale), sputum volume and purulence significantly reduced in both groups after 12 months. FEV1 changes in both groups were unreliable. The number of exacerbations in CLA group reduced from 4.3±0.2 to 1.4±0.2, and in AMC group – from 4.5±0.3 to 2.4±0.3 per year. Exacerbation-free interval was longer in CLA group 258.4±17.0 days than in AMC group 192.8±24.8 (p<0.03).
Conclusion: long-term courses of clarithromycin for the COPD treatment are safe with few mild adverse events. Changes of dyspnea, spirometry parameters, sputum volume and purulence in CLA-treated and AMC-treated patients were equivalent. Long-term clarithromycin treatment of stage II COPD patients with frequent exacerbations was

associated with longer exacerbation-free interval compared to standard AMC treatment.

P849 **Empirical first-line antibiotic-therapy in adult patients with acute upper respiratory tract infections in eastern part of Poland**

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Objectives: The main objective of the study was the evaluation of the frequency and the verification of appropriateness of the use of antibiotics in empirical first-line therapy in adult patients with acute upper respiratory tract infections. In addition, the aim of the study was to discover whether there are any differences in the amount of antibiotics prescribed according to the place of residence (urban, rural), gender and age.
Methods: The analysis covered medical records of 4,047 patients from 47 health centres in the Lublin Region (eastern part of Poland) concerning the period of one year – 1 September 2005 – 31 August 2006. From among all the diagnoses, only newly diagnosed cases of acute upper respiratory tract infections were selected in patients who had no medical history of chronic respiratory diseases. Age, gender, and place of residence of patients were recorded, as well as the fact of prescribing or not prescribing an antibiotic by a physician during the first visit, and the type of antibiotic used.
Results: The documentation contained the records concerning 1,386 visits, the reason for which was qualified by physicians as acute infection of the upper respiratory tract. Acute pharyngitis and tonsillitis, acute respiratory tract infections of multiple and unspecified sites, a common cold, and acute bronchitis were the most frequent diagnoses made. While analysing all the cases it was noted that antibiotics were applied in empirical first-line therapy in 1,103 cases, which constituted 79.58% of the total number of morbid cases. The antibiotics most frequently used were: amoxicillin (26.77%), amoxicillin with clavulanic acid (13.1%), macrolides (12.5%), and doxycycline (10.5%). No statistically significant differences in the use of antibiotics were observed according to patients' age, gender, and place of residence.
Conclusions: The analysis showed that in the great majority of cases the use of antibiotics was inappropriate. If this situation persists, in the near future it will lead to an increase in the phenomenon of antibioticoresistance in Poland. In order to prevent this situation, it is necessary to carry out an extensive educational action concerning the hazardous effect of excessive use of antibiotics, both among physicians and the whole society.

Methods for antibiotic susceptibility testing

P850 **Investigative use only test card using the VITEK 2 system as compared to broth microdilution**

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Objective: The purpose of this study was to demonstrate the effectiveness of antimicrobial susceptibility tests for Cefalexin (CN), Cefditoren (CDN), Cefsulodin (CFS), and Ceftobiprole (BPR) on the VITEK® 2 platform.
Methods: This multicentre trial tested Gram-negative isolates using an investigative use only (IUO) card (GN14) containing CN, CDN, CFS, and BRP. Challenge isolates (100), reproducibility isolates (best 10 strains), and quality control isolates (20 replicates of *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922) were tested at each site by automatic and manual dilution using the GN14 IUO card. Clinical isolates were tested by automatic dilution at all sites. Broth microdilution (BMD) reference testing was performed on all reproducibility, challenge, quality control and clinical isolates using the same standard inoculums prepared for the GN14 test cards. Reproducibility tests were performed in triplicate daily for three days. Essential agreement (EA) (within ± one log dilution) and categorical agreement (CA) (S/I/R) was determined for each microorganism/antimicrobial combination.
Results: Essential agreement for all 431 clinical isolates was > 93.0%, while categorical agreement was > 90.3% (Table 1). Reproducibility for the automatic diluted isolates was 100.0%, 98.9%, 97.8% and 98.9% for CN, CDN, CFS and BRP, respectively. For the manual dilution, reproducibility was 98.9%, 98.9%, 92.2% and 98.9% for CN, CDN, CFS and BRP, respectively. The EA, for 100 challenge experiments using automatic and manual dilution methods, was >96.0% for all antibiotics.

Table 1: Percent EA and CA for GN14 Card vs. BMD

Type of isolates		CN	CDN	CFS	BRP
Clinical (N = 431)	EA	93.0	97.7	96.8	97.2
	CA	96.3	90.3	95.8	95.4
Challenge Automatic Dilution (N = 100)	EA	100.0	99.0	97.0	100.0
	CA	99.0	93.0	95.0	93.0
Clinical & Challenge Auto Dilution (N = 531)	EA	94.4	97.9	96.8	97.7
	CA	96.8	90.8	95.7	94.9
Challenge Manual Dilution (N = 100)	EA	99.0	99.0	96.0	100.0
	CA	99.0	91.0	94.0	93.0

Conclusions: This evaluation provides convincing evidence that the performance of antimicrobial susceptibility tests for CN, CDN, CFS and BRP on the VITEK® 2 platform is comparable to conventional testing in a clinical laboratory.

P851 **Comparison of the VITEK®2 AST-N054 test card with MASTDISCS® manual disc testing for the detection of extended spectrum β-lactamase production in *Escherichia coli* with CTX-M phenotypes**

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Objectives: A new antibiotic susceptibility card, AST N-054, was introduced for testing aerobic Gram-negative bacilli on VITEK®2 (bioMerieux) systems in 2007 and has been widely adopted for routine use in the UK. We compared its performance to an established manual method for detecting extended-spectrum β-lactamase (ESBL)-production in *Escherichia coli*. *E. coli* with CTX-M enzymes are now prevalent throughout the UK, partly owing to the spread of epidemic clones with CTX-M-15 enzyme.
Methods: ESBL-producing faecal isolates of *E. coli* (n = 337) recovered from patients in nursing homes were evaluated. Based on greater resistance to cefotaxime than ceftazidime in agar dilution, all were inferred to have CTX-M type enzymes. Seventy-three belonged to strain A, the most prevalent epidemic lineage, which typically produces a low level of CTX-M-15 enzyme; 64 were non-strain A and their CTX-M enzymes remain to be fully identified. Each isolate was tested using the AST N-054 card on the VITEK®2 and with MASTDISCS® ID ESBL detection disc diffusion (DD) tests (Mast Diagnostics). All VITEK®2 advanced expert system (AES) summary interpretations that included 'ESBL' were categorised as successful. The susceptibility result recommended by the AES software was also recorded.
Results: Overall, the AST N-054 card detected ESBL production in 93 of the 137 isolates tested (test sensitivity 67.9% [95% CI, 59.7–75.1]). Strain A accounted for the majority of the 44 detection failures, with 35/73 strain A isolates incorrectly reported vs 9/64 non-strain A isolates ($p<0.0001$). By comparison, manual detection based on UK national recommendations with screening using cefotaxime and ceftazidime discs followed by confirmation using the MASTDISCS® method correctly detected ESBL in 135 of the 137 isolates (test sensitivity 98.5% [95% CI, 94.5–99.9]). Of the 44 isolates found to be negative for ESBL production by VITEK®2, the AES reported 29 as susceptible to cefotaxime, and all as susceptible to ceftazidime and aztreonam.
Conclusion: These data suggest that the AST N-054 card for the VITEK®2 system may be less reliable than combination discs for

detection of CTX-M β-lactamase-producing *E. coli* circulating in the UK; particularly for the strain A isolates, which tend to be less resistant to cefotaxime and ceftazidime than most others. In consequence many ESBL producers may be inappropriately reported as susceptible to substrate cephalosporins.

P852 Analysis of the Dade Behring MicroScan Walkaway system for the identification and antimicrobial susceptibility testing of cystic fibrosis *Pseudomonas aeruginosa*

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Objectives: Cystic Fibrosis patients are often colonised with atypical, hypermutatable strains of *Pseudomonas aeruginosa* (CF-PSA). The ability of CF-PSA to mutate frequently promotes diversification within the CF lung resulting in multiple colonial morphotypes and conferring adaptive mutations such as loss of certain phenotypic characteristics and resistance to antimicrobials. Consequently it is unsurprising that CF-PSA are notoriously difficult both to identify and to assess for their antimicrobial susceptibility.
This evaluation assessed the ability of the Dade-Behring Walkaway system to correctly identify and accurately assess antimicrobial susceptibility of CF-PSA isolates by comparison with BSAC disc diffusion methodology.
Methods: 58 CF-PSA isolates of mixed morphotype inocula were used. These were tested using the Miscroscan NC-32 panel. Discrepancies in identification were further investigated using API 20NE. Five antimicrobial agents were compared with results interpreted using BSAC breakpoint guidelines.
Results: 66% of isolates identified correctly with a high probability (>85% likelihood). 15% identified correctly at low probability, the remainder (19%) either gave an incorrect identification with a low probability or failed to give an identification.
A total of 290 antimicrobial agent/isolate combinations were tested of which 240 (83%) were in complete agreement. The overall false resistant (major) error rate was 17% (50/290). No false susceptible (very major) errors were observed.
Conclusion: CF-PSA showed atypical reactions for several identification tests; notably only 69% of all isolates were positive for citrate utilisation, and 66% were positive for tobramycin resistance. Five of the identification tests within the panel are tests for antimicrobial resistance. Given that the levels of resistance in CF-PSA are unusually high, it seems inappropriate for susceptibility to be used as a means to achieve identification of these organisms.
In our hands for our CF-PSA isolates the system demonstrated apparently high false resistant rates.
It was noted however that many of the isolates that were resistant to meropenem demonstrated inducible resistance to other β-lactams. It may be that the Walkaway was able to detect resistant mutants that could not be discerned using BSAC guided disc diffusion and this area may warrant further investigation.

P853 Comparison of three automated systems (BD Phoenix, MicroScan WalkAway and VITEK 2) for detecting imipenem resistance in *Acinetobacter baumannii*

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Objectives: Carbapenems are considered the gold standard treatment for multidrug resistant *Acinetobacter baumannii* infections, however, resistance to this agent has been reported increasingly. Reliable susceptibility testing results remains a critical issue for the clinical outcome for these patients. Automated systems are currently used in many clinical laboratories. This study was organised to evaluate the accuracies of three automated susceptibility testing methods for testing *Acinetobacter baumannii* against imipenem.
Methods: One hundred twelve selected isolates of *Acinetobacter baumannii* collected between January 2003 and December 2006 were tested to confirm imipenem susceptibility results. Bacterial isolates were identified according to conventional bacteriological techniques and confirmed by API 20 NE (bioMerieux, France). Strains were tested against imipenem by broth dilution, disk difusion, Etest (AB Biodisk, Sweden), BD Phoenix (Becton Dickinson), MicroScan WalkAway (Dade Behring) and VITEK 2 (bioMérieux) automated systems. Data were analysed by comparing the results from each test method to those produced by the reference broth microdilution (BMD) test performed using in-house prepared panels.
Results: MicroScan performed true identification of all *A. baumannii* strains while VITEK 2 unidentified one strain, and Phoenix unidentified two strains and misidentified two strains. The BMD testing showed that 25 strains were susceptible to imipenem while 87 were resistant All other test systems in the study produced errors when *A. baumannii* was tested against imipenem. Etest showed the best performance with only two minor error (1.8%). VITEK 2 produced eight minor errors(7.2%). BD Phoenix produced three major errors(2.8%). Disk diffusion produced two very major errors (1.8%) and three major errors (2.7%). MicroScan showed the worst performance in susceptibility testing with 28 very major errors (25%) and 50 minor errors (44.6%).
Conclusions: Testing difficulties for imipenem do exist in susceptibility testing systems. Reporting errors can have serious implications for the clinical outcome for patients. We suggest clinical laboratories using automated systems for routine use should consider using a second, independent antimicrobial susceptibility testing method to validate imipenem susceptibility.

P854 Evaluation of the BD Phoenix™ Automated Microbiology System to detect inducible-macrolide-lincosamide-streptogramin B resistance in *Staphylococcus* species

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Objectives: Macrolide-resistant (Mac-R) staphylococci may have constitutive or inducible resistance to clindamycin. Currently, constitutive resistance to clindamycin can be easily detected by a number of manual and automatic antimicrobial susceptibility testing methods. On the other hand, inducible resistance to clindamycin can only be detected by the D-test as described by CLSI. The D-test requires placing a 2-ug clindamycin disk and 15-ug erythromycin disk approximately 15 mm apart per standard disk diffusion testing methods. Mac-R staphylococci showing the “D-zone” would be reported as clindamycin-resistant. In this study, we will describe a new integrated test to be offered in the BD Phoenix Automated Microbiology System (BD Diagnostic Systems, Sparks MD) for the automatic detection of inducible clindamycin resistance in staphylococci.
Methods: A total of 408 strains of staphylococci, including 293 *Staphylococcus aureus* and 115 coagulase negative staphylococci were characterised by Standard Broth Microdilution (SBM). All Mac-R staphylococci strains were tested using the D-test. Each Mac-R staphylococci strain was then tested in the Phoenix system using panels with the integrated iMLSb test reagent and algorithm. Workflow and results obtained from the Phoenix method were compared to the CLSI method for accuracy.
Results: Out of the 408 strains tested, 194 strains were determined by SBM as Mac-R and clindamycin susceptible staphylococci. Of these strains, 136 were determined to be inducible-clindamycin resistant by the D-test. The following table compares the Phoenix vs. D-test results. The overall sensitivity and specificity using the Phoenix iMLSb test was 99.3% and 96.6%, respectively.

		D-Test	
		positive	negative
Phoenix iMLSb	positive	135	2
	negative	1	56

Conclusions: The integrated iMLSb test in the Phoenix System provides accurate detection of inducible-clindamycin resistance in staphylococci. This method also eliminates the necessity of performing the D-test and the associated subjective interpretation of test results.

P855 Identification and susceptibility testing of *Enterococcus casseliflavus* and *Enterococcus gallinarum* by Phoenix 100 system (Becton Dickinson)

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Objectives: Although *E. casseliflavus/flavescens* and *E. gallinarum* are of low pathogenic potential, they may be implicated in invasive infections. The aim of the present study was to assess their correct recognition by Phoenix and to investigate their antimicrobial susceptibility profile using the same system.
Methods: A total of 82 consecutive non-*E. faecalis* and non-*E. faecium* enterococcal isolates were collected during two hospital surveillance studies for VRE faecal carriage; all were identified as *E. casseliflavus/gallinarum* by Phoenix (without species discrimination). Speciation was confirmed by a multiplex PCR assay (specific for ddl-*E. faecium*, ddl-*E. faecalis*, vanA, vanB, vanC1/2, vanD, vanE and vanG genes). Pigment production was also recorded. Susceptibilities towards 8 antimicrobials (penicillin, ampicillin, erythromycin, gentamicin, streptomycin, linezolid, teicoplanin and vancomycin) were determined by Phoenix and E-tests (AB, Biodisk) against linezolid and teicoplanin were performed for selected isolates.
Results: According to PCR all 82 isolates harboured the vanC ligase gene and by pigment production 66 were identified as *E. gallinarum* and 16 as *E. casseliflavus/flavescens*. All but one isolates tested susceptible to both penicillin and ampicillin. Eleven strains (13.4%) were found to be intermediately resistant and 5 (6.1%) resistant to erythromycin. Five isolates (6.1%) exhibited resistance against streptomycin. For 3 *E. gallinarum* isolates Phoenix produced a teicoplanin MIC >16 μg/ml, not confirmed by the E-test and PCR (3 major errors, 3.6%). Vancomycin MICs by Phoenix ranged from 4 to >16 μg/ml. Regarding linezolid, Phoenix characterised 6 strains as resistant (all susceptible by E-test; at least 6 major errors) and 21 strains as intermediately resistant (only 4 intermediately resistant by E-test; at least 17 minor errors). On repeat testing Phoenix classified again 8 susceptible isolates as intermediately resistant and 1 intermediately resistant strain as susceptible (at least 9 minor errors).
Conclusion: *E. gallinarum* and *E. casseliflavus/flavescens* are accurately identified as a group by Phoenix and are rather multi-susceptible species with the former being more frequently recovered from faecal samples of hospitalised patients. Rare major errors with teicoplanin and major and minor errors with linezolid, which may call into question correct speciation, stress the need for confirmation with supplementary testing.

P856 Evaluation of the Oxoid M.I.C.Evaluator susceptibility test strip in comparison to Etest assay and BSAC agar dilution

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Objectives: The Oxoid M.I.C.Evaluator™ strip comprises an antibiotic gradient immobilised on a plastic support strip. We compared its performance with AB Biodisk's Etest® product and with the BSAC agar dilution method, which was taken as a reference.
Methods and Materials: All tests, whether by M.I.C.Evaluator strip, Etest strip, or agar dilution were done on IsoSensitest agar, supplemented as recommended for agar dilution by the British Society for Antimicrobial Chemotherapy. The inocula used for both strip types corresponded to a 0.5 McFarland. A total of 8775 individual organism/antibiotic combinations were tested by each method; the total number of isolates was 837, chosen to represent most clinically relevant fastidious and non-fastidious species.
Results: Essential agreement of MIC values, ± 1 doubling dilution to the agar dilution reference method, with off-scale results excluded, was 88.2% for the M.I.C.Evaluator strip against 86.0% for the Etest strip. When one of more value was off-scale, (e.g. strip >32 mg/L and agar dilution 128 mg/L) agreement was 72.4% for the M.I.C.Evaluator strip against 69.9% for the Etest strip; this included instances, (e.g. cefotaxime and oxacillin vs. MRSA) where MICs are notoriously fickle. For both strip types, agreement was best for non-fastidious genera including staphylococci, enterococci, Enterobacteriaceae and non-fermenters and weaker for beta-haemolytic streptococci and *H. influenzae*. In many instances where there was disagreement with the reference method, the results of the M.I.C.Evaluator and Etest strips agreed with each other, whether for individual isolates, or for particular antibiotics against the generality of a species: for example, both tended to estimate lower imipenem MICs than agar dilution for *P. mirabilis*.
Conclusion: The M.I.C.Evaluator™ strip has essentially equivalent performance to the Etest® strip, with both giving good agreement to agar dilution results determined by the BSAC method.

P857 Performance evaluation of M.I.C.Evaluator™ strip (Oxoid) and comparison with the BSAC reference method

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Objectives: To evaluate the performance of a new product, the M.I.C.Evaluator™ strip for the determination of minimum inhibitory concentrations (MIC) of antibiotics for a selection of reference strains, recent isolates and stored strains displaying a variety of resistance mechanisms.
Methods: The M.I.C.Evaluator™ strip was tested in parallel with the BSAC agar dilution reference method. Enterobacteriaceae, *Pseudomonas*, *Acinetobacter*, staphylococci, streptococci, enterococci, *Haemophilus* and *Neisseria* together with appropriate reference strains were tested. Antibiotics were as shown in the results table. Iso-Sensitest Agar with or without supplements was the basic medium. Inocula were standardised to 0.5 MacFarland, strips were placed on 90 mm dishes and incubated at a temperature of 35–37°C for 18–20 h or, for some strains, up to 24 h. The reference method was carried out according to BSAC guidelines. Results were read and recorded by different workers working independently.
Results: For each organism group/antibiotic combination the middle figure shows the essential agreement (EA) i.e. ± one dilution step, between results obtained with M.I.C.Evaluator™ strip and the reference method. The figure to the left shows the percentage yielding results two or more dilution steps lower than the reference method and the figure to the right shows the percentage of results two or more dilution steps greater than the reference method. All ATCC reference strains gave MIC results within the published range.

Antibiotic n =	Enterobacteriaceae 202	*Pseudomonas* 66	*Acinetobacter* 34	*Haemophilus* 53	*Staphylococcus* 153	*Streptococcus* 163	*Neisseria* 55	*Enterococcus* 102
Amoxy/clav	0–100–0			2–98–0				
Amoxycillin	0–84–16			4–96–0		1–97–2		
Ampicillin	0–95–5			0–96–4		1–99–0	9–89–2	0–85–15
Cefotaxime low	0–87–13			0–81–19		1–63–36	0–84–16	
Cefotaxime high	0–77–23			0–79–21		0–61–39	0–91–9	
Ciprofloxacin	0–70–30	0–97–3	0–97–3		0–92–8	0–100–0	0–76–24	
Erythromycin				0–100–0	0–93–7	4–93–3		
Gentamicin low	0–93–7	0–64–36	0–100–0			0–95–5		
Gentamicin high	0–95–5	0–73–27	0–91–9			1–92–7		
Imipenem	0–97–3	0–100–0	0–100–0	13–87–0		5–91–4		0–100–0
Linezolid					0–99–1	20–80–0		3–97–0
Levofloxacin	0–96–3			0–100–0	0–86–14	1–98–1		
Oxacillin					1–85–14	5–87–8		
Penicillin low					3–46–51	1–73–26	0–95–5	
Penicillin high					3–58–39	1–74–25	0–95–5	
Tetracycline				6–94–0	1–92–7	12–88–0	13–87–0	
Vancomycin						3–96–1		

Conclusion: M.I.C.Evaluator™ strip compared to BSAC reference method showed >80% EA for ampicillin, amoxicillin, amoxicillin/clavulanic acid, erythromycin, imipenem, levofloxacin and vancomycin. In almost all instances of poor agreement the M.I.C.Evaluator™ gave a result greater than that of the reference method.

P858 Comparison of Oxoid M.I.C.Evaluator™ device with broth micro-dilution and Etest® device from AB Biodisk for antimicrobial susceptibility testing of staphylococci, streptococci and enterococci

R. Rennie, L. Turnbull, C. Brosnikoff (Edmonton, CA)

Objective: The current study was designed to evaluate a new product utilising an agar based gradient endpoint system (MICE™) produced by Oxoid (Thermo Fisher Scientific) as an alternative to broth microdilution for accurate MIC test results of staphylococci, streptococci and enterococci that can be utilised effectively in critical care.
Methods: A total of 154 strains of MSSA, MRSA and CNS, 158 strains of *S. pneumoniae*, beta-haemolytic streptococci and *viridans* group streptococci, and 106 strains of enterococci were tested. A total of 13 antimicrobial agents that were evaluable for each genus and species were tested. The strains were inoculated into BMD trays (CLSI M7-A7) and onto Mueller Hinton agar. The MIC strips were added to the plates according to each manufacturer's instructions and were incubated for 20–24 hr at 35°C. Inhibition was read where the elliptical zone intersected with the strips. ATCC *S. aureus* 29213, *S. pneumoniae* 49619, and *E. faecalis* 29212 were the quality control strains.
Results: Quality control strains were within CLSI published ranges. For the clinical strains, 5300 agent-organism MIC results were available for analysis. Over 99% of results were within 2 doubling dilutions comparing BMD to either Oxoid's MICE™ or AB Biodisk's Etest® device. Compared to BMD there were 5 Very Major errors (0.09%), and 8 Major errors (0.15%) observed with both devices. Four of the 8 major errors occurred with penicillin and staphylococci
Conclusions: MICE™ device results for clinical strains of staphylococci, streptococci and enterococci were comparable to the gold-standard CLSI BMD observations and to Etest® device results for 13 evaluable antimicrobial agents. Additional antimicrobials are undergoing evaluation. The gradient endpoint MIC device provides accurate susceptibility test results for these Gram-positive clinical species.

P859 Comparison of Oxoid M.I.C.Evaluator™ device with broth micro-dilution and Etest® device from AB Biodisk for antimicrobial susceptibility testing of Enterobacteriaceae

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Objective: The current study was designed to evaluate a new product utilising an agar based gradient endpoint system (MICE™) produced by Oxoid (Thermo Fisher Scientific) as an alternative to manual and automated broth microdilution systems for accurate MIC test results of Enterobacteriaceae that can be utilised effectively in critical care.
Methods: 250 recent clinical strains of Enterobacteriaceae, including 16 genera and over 30 species were tested. Initially, seven evaluable antimicrobials were tested: ciprofloxacin, amoxicillin, amoxicillin-clavulanate, ampicillin, levofloxacin; and cefotaxime and gentamicin (low and high concentrations). The strains were inoculated into BMD trays (CLSI M7-A7) and onto Mueller Hinton agar. The MIC strips were added to the plates according to each manufacturer's instructions and were incubated for 20–24 hr at 35°C. Inhibition was read where the elliptical zone intersected with the strips. ATCC *E. coli* 25922 *P. aeruginosa* 27853, *S. aureus* 29213 and *E. faecalis* 29212 were the quality control strains.
Results: Quality control stains were within CLSI published ranges. For the clinical strains, 2250 agent-organism MIC results were available for analysis. Over 98% of results were within 2 doubling dilutions comparing BMD to either Oxoid's MICE™ or AB Biodisk's Etest® device. Compared to BMD there were 8 Very Major errors (0.4%), 1 Major error (0.04%) and 7 minor errors (0.3%) observed with both devices. Six of the 8 VM errors occurred with amoxicillin and ampicillin with *Proteus* species.
Conclusions: MICE™ device results for clinical strains of Enterobacteriaceae were comparable to the gold-standard CLSI BMD observations and to Etest® device results for seven evaluable antimicrobial agents. Additional antimicrobials are undergoing evaluation. The gradient endpoint MIC device provides accurate susceptibility test results for Enterobacteriaceae.

P860 Validation of M.I.C. Evaluator strips following ISO guidelines

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Objectives: Oxoid has developed a new system for determining the MIC. These M.I.C.Evaluator strips (M.I.C.E.) provide a gradient of antibiotic stabilised on a polymer strip covering 15 doubling dilutions.
The aim of the study was to evaluate these M.I.C.E. vs the MIC reference method (ISO 20776–1) according to ISO guidelines (ISO 20776–2).
Methods: Following the ISO protocol, during daily clinical laboratory work 300 consecutive Gram-negative strains (fresh isolates) were collected. Identification was performed following standard procedures. From each strain, the MICs were determined following ISO guidelines (ISO 20776–1) and instructions of the M.I.C.E. manufacturer, respectively. For the M.I.C.E. an inoculum of 0.5 McFarland was streaked on a Mueller-Hinton agar plate. The M.I.C.E. were put on the plate and incubated for 16–20 h. The MIC values were read following instruction by two independent lab technicians. The following M.I.C.E. were evaluated: gentamicin 256–0.016 mg/L (Ge256), gentamicin 1024–0.064 mg/L (GE1024), amoxicillin 256–0.016 mg/L (AM), cefotaxime 32–0.002 mg/L (Cef32), cefotaxime 256–0.016 mg/L (Cef256), levofloxacin 32–0.002 mg/L (LE), imipenem 32–0.002 mg/L (IM), ciprofloxacin 32–0.002 mg/L (CI). For the interpretation of the MIC and M.I.C.E. the EUCAST breakpoints were used. Discrepancy analysis was performed following the ISO guideline.
Results: The isolates collected belonged to the following species *Citrobacter* spp. (n = 35), *Enterobacter* spp. (n = 35), *Escherichia coli* (n = 30), *Hafnia alvei* (n = 3), *Klebsiella* spp. (n = 34), *Morganella morganii* (n = 30), Pantoea agglomerans (n = 3), *Proteus* spp. (n = 37), *Providencia rettgeri* (n = 3), *Serratia marcescens* (n = 3). 63% of the strains were susceptible for the different antimicrobial agents. The category agreement after discrepancy analysis was: Ge256 99%, GE1024 99%, AM 96%, Cef32 99%, Cef256 99%, LE 98%, IM 99% and CI 98%. Very Major Discrepancy was found only for CI 1%, Cef32 0.7% and Cef256 0.3%; and none for the other antimicrobial agents. There was no Major Discrepancy was for any antimicrobial agents.
Conclusion: The new M.I.C.E. to determine MICs demonstrated an excellent performance for the interpretive category determinations of SIR of the antimicrobial agents tested. M.I.C.E. provide a welcome new methode to determine susceptibility during routine practice.

P861 Effect of lower inoculum on MIC broth microdilution: a comparison with the ISO reference method

M.H. Nabuurs-Franssen, M.T.H. Bohne, J.W. Mouton (Nijmegen, NL)

Objectives: Rapid, accurate and reproducible determination of the MIC value is important for adequate patient care, for local and international resistance surveillance. For epidemiological studies of susceptibility and for comparison of new and existing antimicrobial agents. The ISO reference method (ISO 20776–1) prescribes an inoculum of $2–8\times10^5$. The rationale behind the lower limit is not fully clear. In addition, some MIC devices do use lower inocula. For instance, the (former) guideline of the BSAC stated 10^5 /ml. The aim of this study was to evaluate the effect of a lower inoculum size on the MIC determination using the ISO protocol (ISO 20776–2).
Method: During daily clinical laboratory work 300 consecutive Gram-negative strains (fresh isolates) were collected. Identification was performed following standard procedures. From each strain, the MICs

were determined with both the 2–8×10^5 inoculum (ISO 20776–1) and with a 5–10×10^4 inoculum. MIC values were read independently by two lab technicians. For the interpretation of the MICs the EUCAST breakpoints were used. The following antimicrobial agents were tested: amoxicillin (AM), cefotaxime (Cef), ciprofloxacin (CI), amoxicillin/clavulanic acid (AC). The MIC values of the lower inoculum size was compared to the reference method following ISO guideline (ISO 20776–2).

Results: The isolates collected belonged to the following species *Citrobacter* spp. (n = 35), *Enterobacter* spp. (n = 35), *Escherichia coli* (n = 30), *Hafnia alvei* (n = 3), *Klebsiella* spp. (n = 34), *Morganella morganii* (n = 30), Pantoea agglomerans (n = 3), *Proteus* spp. (n = 37), *Providencia rettgeri* (n = 3), *Serratia marcescens* (n = 3). Forty-five percent of the strains were susceptible for the different antimicrobial agents. Following ISO guidelines the essential agreement after discrepancy analysis was: AM 99%, Cef 98%, CI 98%, AC 98% and the category agreement was: AM 96%, Cef 99%, CI 97.%, AC 92%, respectively. The Very Major Discrepancy was found only for Cef 0.3%. Discrepancy analyses with the M.I.C.E. found the same MIC as the 5–10×10^4 inoculum. A Major discrepancy was only found for Cef 0.3%. Minor discrepancies were found for AM 4%, Cef 0.6%, CI 3.3%, AC 8.3%.

Conclusion: In this study, a lower inoculum size (5–10^4) did not influence the outcome of the MIC determination; there was a very good category agreement and essential agreement between the different inoculum sises for the antimicrobial agents tested compared to the reference method.

P862 Detection of VISA/hVISA in routine clinical isolates: Microscan® modified procedure vs. Etest®

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Objectives: Detection of VISA/hVISA strains should be pursued in routine clinical microbiology, to prevent possible therapeutic failure. Although PAP-AUC is known as reference method, many laboratories use more manageable techniques, primarily Etest® (AB-Biodisk). In this work we evaluate the performance of MicroScan® (Dade-Behring) automated method (also with a modified procedure) vs. Etest.

Methods: MRSA clinical isolates, mostly from SSI, BSI and ulcers of hospitalised patients in Italy (8 centres in Rome) in 2007, not classified by respective centres as VISA/hVISA (vancomycin MIC ⩽2 μg/ml at Vitek® or Phoenix® ASTs for all strains), were retested as follows: culturing on VA agar screen (vancomycin 6 μg/ml – Biolife - 0.5 and 2 McF, 48 h); vancomycin's MIC measure for screened colonies by means of Etest 'standard' (MHA 0.5 McF, 48 h), Etest 'macro method' (BHI 2 McF, 48 h) and Microscan panels (standard 0.5 and modified 2 McF inocula, 48 h).

Results: After screening, 106 collected strains produced 53 suspected 'not-VSSA' (none detected by respective Vitek/Phoenix ASTs). With CLSI 2006 breakpoints, Microscan showed high sensitivity (100%; Wilson CI 75–99), low specificity (55%; CI 38–71), high npv (100%; CI 87–99) and low ppv (47%; CI 29–65) vs. Etest (standard inoculum 0.5 McF, both with Etest and Microscan). Using Walsh's (2001) classification, we obtained similar results: sens.100% (CI 60–99), spec.27% (CI 15–42), npv100% (CI 89–99), and ppv19% (CI 10–35): in this case, 2 McF inoculum was used both for 'macromethod' and Microscan. Correlation (Spearman) was also observed between Microscan and Etest MICs/results: R=0.650 ($p<0.001$) and R=0.512 ($p<0.001$), respectively for 0.5 and 2 McF.

Conclusion: Microscan seemed to be an alternative to Etest for detection of presumptive VISA/hVISA, even when instrument is forced to work likewise an Etest macromethod. High sensitivity, with advantage of automation, allows practical first level identification of strains. On the other hand, overall data show that use of a glycopeptide agar (VA or TP) for screening is essential for recovery of suspected strains, especially in hospital settings as those analysed, with probable significant unrecognised rate of VISA/hVISA.

P863 Mecillinam susceptibility in *Escherichia coli* with and without ESBL: a comparison of disk diffusion and Etest on Mueller-Hinton agar and Iso-Sensitest agar

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Objective: Mecillinam is a 6-amidinopenicillin highly active against Gram-negative bacteria such as *Escherichia coli* and *Klebsiella pneumoniae* and has been used successfully in Scandinavia for the treatment of uncomplicated urinary tract infections. Mecillinam is semi-stable to many β-lactamases but MIC values for β-lactamase producing strains are elevated to varying degrees. Currently used MIC breakpoints for mecillinam and *E. coli* in Europe are 8/8 (BSAC), 2/8 (CA-SFM), 2/8 (NWGA) and 1/8 (SRGA). This study compared susceptibility results with Etest and disk diffusion on Mueller-Hinton agar (MHA) and Iso-Sensitest Agar (ISA) in *E. coli* strains with and without β-lactamases, including a variety of ESBLs.

Methods: Mecillinam Etest (AB Biodisk, Solna, Sweden) and disks (10 μg, Oxoid, Basingstoke, UK) were used on ISA (Oxoid) and MHA (Oxoid). All testing was performed with Kirby-Bauer inoculum, except for disk diffusion on ISA which was done with a semi-confluent inoculum (as described in the methods by BSAC and SRGA). A total of 280 *E. coli* isolates from urine samples were investigated. Of these, 161 were consecutive strains from Kronoberg County and 119 were ESBL-producers from Karolinska University Hospital. ESBL production was detected by clavulanate synergy disks (Oxoid), followed by PCR targeting CTX-M, TEM and SHV β-lactamases, and subsequent sequencing.

Results: There was excellent correlation between susceptibility testing performed on ISA and MHA, with r>0.99 for MIC v MIC both for the consecutive *E. coli* and the ESBLs. The zone diameters were generally smaller on MHA, but again correlation was strong (r=0.93 for the consecutive *E. coli* and r=0.97 for the ESBLs). The distribution of mecillinam MIC from our consecutive *E. coli* was closely similar to that presented on the EUCAST website with a peak at 0.125 mg/L. The ESBL-producing *E. coli*, mostly CTX-M-15 and -14, had higher MIC values with a peak at 0.5–2 mg/L. EUCAST has suggested a preliminary MIC breakpoint of S ⩽ 8, R>8 mg/L. Our results indicate a tentative disk diffusion breakpoint for a mecillinam 10 μg disk of S ⩾15 mm, R ⩽14 mm, both for MHA and ISA.

Conclusion: There was good agreement between MIC and disk diffusion for mecillinam and *E. coli* on both ISA and MHA. We suggest that disk diffusion can be used to predict mecillinam susceptibility for *E. coli* strains both with and without ESBL enzymes.

P864 Comparison of Etest and combined disk for detection of metallo-β-lactamase-producing isolates

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Objective: The aim of this study was to comparatively evaluate the ability of E-test MBL strip (AB BIODISK, Solna, Sweden) and combined disk (CD) assay for detection of MBL-producing *Pseudomonas aeruginosa* (PSA), *Acinetobacter* spp. (ACB) and enterobacterial clinical isolates.

Methods: A total of 46 genetically unrelated clinical isolates were tested, including 28 *P. aeruginosa* (10 SPM-1, 14 IMP-type, 3 VIM-type and 1 GIM-1), 1 P. putida (IMP-1), 10 *Acinetobacter* spp. (9 IMP-1, 1 SIM-1), and 7 enterobacterial strains [*Enterobacter cloacae* (1 IMP-1 and 3 VIM-1), *Klebsiella pneumoniae* (2 IMP-1) and *Serratia marcescens* (1 IMP-1)]. Nineteen imipenem (IMI)-resistant isolates but non-MBL producers were included as negative controls. The isolates were classified as MBL producers by E-test according to the manufacturer's instructions. The CD assay was performed adding 2, 4, 6, 8 and 10 ul of EDTA (100 mM) to IMI disks. The isolates were categorised as MBL producers considering an increase of ⩾5 mm in inhibition zone of IMI/EDTA disks, compared to the IMI disk alone.

Results: The sensitivity (S) and specificity (E) of E-test and CD assay are shown in the table below. Results of S and E (100% and 33.3%, respectively) for E-test were the same regardless of the MBL type produced. The best E results obtained by Etest and CD were observed for the Enterobacteriaceae group (80% and 100%, respectively). Two positive controls (*Acinetobacter* spp.) and four negative controls (2 *P. aeruginosa* and 2 enterobacterial strains) were categorised as non determinable (ND) by E-test. The best S and E results for CD assay were achieved after dropping 6 ul of the EDTA.

	E-test®		CD	
Species	S %	E %	S %	E %
General	100	33.3	76	95
Acinetobacter spp.	100	0	40	50
P. aeruginosa	100	25	93	83
Enterobacteriaceae	100	80	100	100

S: sensitivity; E: specificity.

Conclusions: Our results indicated that, although the E-test was able to correctly identify almost all MBL-producing isolates, false positive MBL results may occur when using this methology. On the other hand, false negative results may arise when CD test is used to screen for MBL-producing isolates. In summary, these two methods are suitable for preliminary MBL screening for some selected species, but should not be used as the unique indicator of such resistance determinants production.

P865 Comparison of Neo-Sensitabs™ with paper discs in the routine disc diffusion antimicrobial susceptibility testing

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Objectives: The aim of this study was to compare the use of Neo-Sensitabs™ (Rosco, Taastrup, Denmark) with paper discs for antimicrobial susceptibility testing by disc diffusion method according to the recommendations of CLSI.

Methods: We analysed antimicrobial susceptibility of 476 clinical bacterial isolates and 7 ATCC control strains by parallel disc diffusion susceptibility testing with paper discs (Oxoid Ltd, UK) and Neo-Sensitabs™ (table 1). Clinical strains were tested once, but testing was repeated when interpretative disagreement was found. ATCC control strains were tested weekly 10 times.

Results: Zone correlation with the Pearson test value for diameters produced by the discs of two manufacturers was high. Overall agreements (test results within 4 mm range) were also very high. When the comparison was based on CLSI breakpoint categorisation, agreement ranged between 78.5% and 100% (table 1).

Very major, major and minor error rates were 0.2% (8 strains), 0.15% (6 strains) and 2.7% (106 strains) of all analyses, respectively. Three out of 8 very major errors occurred when there was no intermediate category. The rest 5 were detected when testing amoxicillin + clavulanic acid (AMC) or cefuroxime (1 case) activity against ESBL-strains. 59% of all errors occurred when testing activity of betalactams (mostly AMC) against ESBL-strains, and that is very understandable considering the character of this kind of betalactamase enzymes.

All MRSAs were found by both oxacillin discs and only 1 was missed by Oxoid cefoxitin disc, but the oxacillin inhibitory zones of the Neo-Sensitabs™ were more difficult to read at MRSA-strains. Oxoid vancomycin discs failed to detect 4 VRE-strains.

Other observations in routine use: Occasional blank paper discs were found (totally resistant at first and fully susceptible at re-testing). No Neo-Sensitabs™ found to be blank. Paper discs were dropped from the plate at about 1–2% of the analyses. The Neo-Sensitabs™ were not dropped. The Neo-Sensitabs™ were broke down at about 1–2% of the analyses, and it sometimes hampered the measurement of the zone sise. The dispenser of the Neo-Sesitabs™ has 7 locus, which makes the edge effect more prominent in some cases.

Conclusion: The zone diameters produced by Neo-Sensitabs™ and paper discs correlate well and showed good interpretative agreement. Therefore, the Neo-sensitabs™ are potential alternative to the paper discs for antimicrobial susceptibility testing by disc diffusion method.

Table 1: Correlations and agreements of the test results for clinical strains

		Agreement		No. of tests performed			
	Pearson correlation coefficient	Overall	based on CLSI breakpoint categorisation	STC	EC	PAE	AGNR
Ampicillin 10 ug	0.992	96.6	100.0		29		
Aztreonam 30 ug	0.980	97.9	93.6			10	37
Cefoxitin 30 ug	0.969	88.9	98.6	144			
Ceftazidime 30 ug	0.954	98.1	94.8			44	169
Ceftriaxone 30 ug	0.981	97.1	95.9				170
Cefuroxime 30 ug	0.968	96.9	96.3				161
Ciprofloxacin 5 ug	0.982	98.6	98.8	130		47	170
Clindamycin 2 ug	0.977	93.5	97.8	139			
Co-amoxiclav 30 ug	0.934	89.0	78.5[1]				172
Co-trimetoxazole 30 ug	0.976	93.4	96.5	150			167
Imipenem 10 ug	0.975	97.2	100.0		92	48	146
Linezolid 30 ug	0.841	98.0	100.0	150	96		
Meropenem 10 ug	0.902	98.0	100.0			25	127
Nitrofurantoin 300 ug	0.956	97.0	90.9		8		37
Oxacillin 1 ug	0.982	95.2	93.9	147			
Piperacillin+tazobactam 110 ug	0.954	98.1	97.6			49	161
Rifampicin 5 ug	0.957	97.2	100.0	145			
Tobramycin 10 ug	0.962	98.8	98.8	135		49	162
Trimethoprim 5 ug	0.976	98.8	99.7	150			173
Vancomycin 30 ug	0.886	100.0	97.1[2]	147	98		

STC = *Staphylococcus* spp., including 34 MRSA strains. EC = *Enterococcus* sp., including 21 VRE strains. PAE = *Pseudomonas aeruginosa*. AGNR = Aerobe Gram-negative rod, including 41 ESBL strains.
1. 95% of disagreements were strains producing ESBL-, AmpC or K1-β-lactamases.
2. 7/8 of disagreements were VRE-strains. In 4 cases Oxoid discs failed to detect VRE. So the rates of true test results considering the resistance mechanism are 99.0% for Rosco tablets and 95.0% for Oxoid discs.

P866 Comparison of two methods (Etest and MicroScan) for detecting vancomycin susceptibility in *Staphylococcus aureus*

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Objective: The efficacy of vancomycin in the treatment of infections due to *Staphylococcus aureus* and specifically to MRSA has come under debate. There is also a concern for the decreasing in vitro susceptibility of isolates to vancomycin, the difficulty in laboratory detection, and the potential therapeutic implications of resistance. Moreover, vancomycin efficacy is lower against MRSA infections with agr group II isolates. In this study we compared two methods for detecting vancomycin susceptibility among clinical isolates of *S. aureus* and determine the agr groups of the MRSA isolates.

Methods: A total of 463 *S. aureus* isolates (135 MRSA) obtained from a national multicentre survey in Spain in a prevalence study performed in 2006 were evaluated. Vancomycin susceptibility was determined using the microdilution method MicroScan (Dade-Behring) and the Etest method (AB Biodisk). The same inoculum was used for both methods. Detection of heterorresistance (GISA/hGISA) was determined by the Etest macrodilution method. A multiplex PCR was used to determine the agr types of the MRSA isolates.

Results: The origins of the isolates were skin/wound (67%), respiratory (13%), blood (8%), urine (6%), and others (6%). A total of 449 *S. aureus* isolates (97%) had vancomycin MICs of less or =1 mg/L, and 14 (3%) had MICs of 2 mg/L with MicroScan. By Etest, 2 isolates had vancomycin MICs of 0.75 mg/L; 58 of 1 mg/L; 242 of 1.5 mg/L; and 161 of 2 mg/L. None of the isolates was GISA/hGISA. Among the MRSA, 129 isolates (95.5%) had vancomycin MICs of less or =1 mg/L, and 6 (4.5%) had MICs of 2 mg/L with MicroScan. By Etest, 1 isolate had a vancomycin MIC of 0.75 mg/L; 3 of 1 mg/L; 67 of 1.5 mg/L; and 58 (43%) of 2 mg/L. Among the MRSA isolates that had a MicroScan result of less or = 1 mg/L, 3% of isolates had both MicroScan and Etest of less or =1 mg/L, 51% had an Etest of 1.5 mg/L, and 46% of 2 mg/L. Of the 6 remaining isolates with MicroScan MIC of 2 mg/L, 2 isolates had an Etest result of 1.5 mg/L, and 4 isolates of 2 mg/L. The agr groups of the MRSA isolates with vancomycin MICs of less or =1 mg/L by MicroScan

were: 17% group I, 80% group II, and 3% group III. All isolates with vancomycin MICs of 2 mg/L by MicroScan were agr group II.
Conclusions: The results of this study show variability in methods for the determination of the in vitro susceptibility of *S. aureus* and MRSA isolates to vancomycin and a high proportion of MRSA isolates belonging to the agr group II.

P867 Re-evaluation by microdilution of Etest method to determine tigecycline susceptibility in *Acinetobacter*

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Objectives: There has been different communications related to not reliability of E-test method with different microorganisms. The aim of this study is to reevaluate by microdilution the presumably resistance to tigecycline (TGC) of 95 Spanish *Acinetobacter* isolates.
Methods: All isolates were derived from different sources. We have collected *Acinetobacter* isolates with a MIC >2 μg/ml, obtained by E-test, from four Spanish hospitals during 6 months. Organism collection, transport and retest of TGC susceptibility was carried out by Laboratories International for Microbiology Studies, a division of International Health Management Associates, Inc. (Illinois. United States). Minimum inhibitory concentrations (MICs) were determined by the CLSI (2007) recommended broth microdilution testing method. TGC was supplied by Wyeth Pharma and all other antimicrobials were supplied by Microscan (Dade Behring, Sacramento, CA). CLSI breakpoints were used for all the antimicrobials. For TGC we used the breakpoints recommended by FDA and EUCAST for Enterobacteriaceae.
Results: Despite of the results obtained by E-test in which all the isolates were resistant to TGC, by microdilution all the isolates were susceptible. TGC was the most potent agent with a MIC50 of 0.5 μg/ml and a MIC90 of 1 μg/ml. One isolate out of 95 had a MIC of 4 μg/ml for TGC. Therefore according to EUCAST and FDA TGC breakpoints, resistance to TGC was 1.1% and 0% respectively. Percentages of resistance to other antimicrobials are shown in the next table.

Antibiotic	No. Resistant isolates	Resistance (%)
Piperacillin-tazobactam	87/95	91.6
Ceftazidime	88/95	92.6
Cefepime	89/95	93.7
Meropenem	86/95	90.5
Levofloxacin	85/95	89.5
Amikacin	69/95	72.6
Minocycline	4/95	4.2
Tigecycline		
EUCAST	1/95	1.1
FDA	0/95	0

Conclusions: TGC was the most active antimicrobial against *Acinetobacter*. It is important to take into account that E-test underestimates in vitro activity of TGC vs. *Acinetobacter*. This could have important implications for patients with an *Acinetobacter* infection.

P868 Vancomycin-resistant enterococci: validation of susceptibility testing and in vitro activity of novel antibiotics

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Objectives: Acquired vancomycin resistance among Danish clinical isolates is rare making detection of low level resistance challenging. We evaluated the current breakpoints, given by EUCAST, ability to discriminate vancomycin resistant enterococci (VRE) from vancomycin susceptible strains. Furthermore we evaluated the antimicrobial activity of daptomycin, linezolid and tigecycline towards both VRE and vancomycin susceptible strains.
Methods: A total of 125 unique isolates were tested. The isolates were classified by PCR as either vanA, vanB or susceptible. They were divided upon 82 *Enterococcus faecium* (33 vanA, 25 vanB and 24 susceptible) and 43 *Enterococcus faecalis* (1 vanA, 20 vanB and 22 susceptible). On each day of testing appropriate QC strains were tested. All determinations were within published QC ranges. MICs were determined with Etest (AB Biodisk) on MüllerHinton (MH) agar according to the instructions of the manufacturer. Disk diffusion test were performed using vancomycin (5 μg.), tigecycline (15 μg.) and linezolid (10 μg.) disks from Oxoid on Iso-sensitest agar, according to the recommendations of the Swedish Reference Group for Antibiotics.
Results: All strains were susceptible to tigecycline (MIC50 and MIC90 were 0.125 mg/L) and linezolid (MIC50 and MIC90 were 2 mg/L). For daptomycin MIC50 and MIC90 were 2 and 4 mg/L respectively. Disk diffusion results were in concordance with Etest results. Vancomycin MIC determination by Etest produced 4 very major errors (resistant strains misinterpreted as susceptible) when compared to the vancomycin genotype. Disk diffusion produced 17 very major errors for *E. faecalis* and 15 for *E. faecium*. A tentative breakpoint of 15 mm. for *E. faecalis* produced only three very major errors while a tentative breakpoint of 16 mm. for *E. faecium* produced only four very major errors. Off note we found that 96% of strains with the vanB gene and 20% of the susceptible strains had a diffuse inhibition zone edge.
Conclusion: Our study suggests that disk diffusion using the current breakpoints is inadequate when testing for vancomycin resistance, producing an unacceptably high number of very major errors. Modifying breakpoints would lessen the number of such errors considerably but not eliminate them. Inspecting the inhibition zone for a diffuse edge may contribute to identifying resistant strains.

P869 Evaluation of phenotypic methods for detection of extended-spectrum β-lactamases in a clinical microbiology laboratory

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Objective: Extended spectrum β-lactamases (ESBLs) are an increasing cause of resistance in nosocomial isolates of Enterobacteriaceae. The laboratory detection of ESBLs can be complex and sometimes misleading. The aim of this study is to evaluate the results of CLSI ESBL disk diffusion screening criteria in 243 *Escherichia coli* and 140 *Klebsiella* spp. nosocomial isolates, together with the results of CLSI disc diffusion clavulanic acid inhibition effect (CAIE), VITEK 2 CAIE and double disk synergy (DDS) methods.
Method: A total of 383 Gram-negative bacilli (243 *E. coli*, 120 *Klebsiella pneumoniae* and 20 *Klebsiella oxytoca*) which were isolated between April 2005-December 2006 and defined as nosocomial pathogens according to CDC criteriae were included in this study. All the strains were evaluated with respect to CLSI screening criteria using the discs; cefpodoxime (POD), ceftazidime (CAZ), cefotaxime (CTX), ceftriaxone (CRO) and aztreonam (ATM). Isolates that were considered to be potential ESBL producers according to the CLSI screening criteriae were then examined with CLSI disc diffusion CAIE using CAZ and CTX in the presence and absence of CA. All the strains were also analysed by VITEK 2 system (AST-GN13 panel, BioMerieux, France) according to MIC breakpoint values of CTX, CTX/CA, CAZ, CAZ/CA, cefepime (FEB), FEB/CA. DDS was tested evaluating the synergistic effect of amoxicilline clavulanic acid (AMC) with CAZ, CTX, CRO, POD and ATM.
Results: According to CLSI screening criteria 148 isolates (91 *E. coli*, 51 *K. pneumoniae*, 6 *K. oxytoca*) were determined as potential ESBL producers. Of these 148 isolates; 139 were DDS positive, 140 were VITEK 2 CAIE positive. Considering the CLSI ESBL confirmation criteria, 134 isolates showed CAIE using CAZ, CAZ/CA and 133 isolates showed CAIE using CTX, CTX/CA. The sensitivities of the five discs used for ESBL disk diffusion screening were as CRO > CTX = ATM > POD > CAZ in decreasing order.

Conclusion: Although phenotypic methods for determination of ESBL production have been widely used in routine microbiology laboratories, they have certain limitations. While the results in this study favour the use of either automated antimicrobial susceptibility system or DDS for this purpose, CLSI phenotypic confirmation with these methods increase the reliability.

P870 A practical aid for detection of extended-spectrum β-lactamases in Enterobacteriaceae

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Objectives: The detection of Extended-Spectrum Beta-Lactamases (ESBLs) is of special importance, because this resistance genes present serious implications for therapy. Currently, genotypic characterisation of ESBL genes is considered the gold standard for ESBL identification. However, the detection of ESBL genes with PCR and sequencing is complex and does not provide information about their expression. In this study, we evaluated the performance of the combination of the molecular hyplex® ESBL ID (h-ES-ID) test and the ESBL screening agar (ESA) in the identification of ESBL-producing Enterobacteriaceae isolates.
Methods: The h-ES-ID is a multiplex-PCR-ELISA for the direct detection of β-lactamase-producing bacteria. In the presence of relevant DNA, specific amplification products of the blaTEM, bla SHV, bla CTX-M (except CTX-M-8) and bla OXA-1 group are synthesised and subsequently visualised with the h-ES-ID hybridisation modules. The ESA is a MacConkey agar with third generation cephalosporins, AmpC β-lactamase inhibitor and vancomycin. We examined a total of 149 clinical Enterobacteriaceae isolates, 61 of them had been previously characterised as ESBL-producers with PCR and sequencing. The other 88 isolates were ESBL negative.
Results: The sensitivity, specificity and duration of test of h-ES-ID and ESA were 98% (60/61), 57% (50/88), 3 hours, 100% (61/61), 93% (82/88) and 18 hours respectively. With the combination of h-ES-ID and ESA, all the ESBL-positive and ESBL-negative isolates were detected correctly. CTX-M ESBL producers were detected within 3 hours with h-ES-ID. The low specificity of h-ES-ID was mainly due to TEM-1 producers among *Escherichia coli* isolates. These isolates were correctly identified with the ESA.
Conclusion: The combination of h-ES-ID and ESA is a quick, effective, and easy to use method for ESBL detection, especially of CTX-M ESBLs, which are the most prevalent ESBLs worldwide. This combination of tests provides useful early therapeutic guidance by the rapid identification of ESBL-positive isolates.

P871 Extended-spectrum β-lactamases screening agar with AmpC inhibition

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Objectives: The serious increase in the prevalence of Extended-Spectrum Beta-Lactamases (ESBLs) worldwide creates a need for effective screening methods to detect these resistance genes. The currently used screening agars have low specificity, mainly due to growth of isolates of species with inducible AmpC β-lactamases. In this study we evaluated an ESBL Screening Agar (ESA) which inhibits growth of AmpC-producing isolates and Enterococci, and compared it with the commercially available BLSE agar (AES Laboratory, France) for selective isolation and presumptive identification of ESBL-producing Enterobacteriaceae.
Methods: The ESA consists of two MacConkey Agars containing either ceftazidime 1.0 mg/l or cefotaxime 1.0 mg/l + cloxacilline 400 mg/l + vancomycine 64 mg/l. The BLSE agar is a commercial double-plate agar (MacConkey + ceftazidime 2 mg/l and Drigalski + cefotaxime 1.5 mg/l). The agars were evaluated with 208 Enterobacteriaceae isolates, 70 of them had been previously genotypically characterised as ESBL-producers. The other 138 isolates were ESBL negative. The ESA was further evaluated with 100 clinical specimens. All clinical specimens were futher characterised for ESBL production with ESBL combined disc test and ESBL Etest.
Results: The sensitivity and specificity of the ESA and the BLSE agar tested with the 208 Enterobacteriaceae isolates were 100% (70/70) and 84.7% (117/138), and 100% (70/70) and 57.2% (79/138) respectively. Isolates of species with inducible AmpC were most commonly the false positives on BLSE agar. The ESA detected all 5 ESBL-positive clinical specimens correctly, 4 specimens were false positive. The sensitivity and specificity of the ESA in the clinical specimens in this study were 100% and 95.7% respectively.
Conclusion: The specificity of ESA for screening of ESBL-producing strains was significantly better than the specificity of BLSE agar; the better performance of the ESA was mainly due to less false positive results due to Amp-C-producing strains. The ESA performed well also when inoculated directly with clinical specimens. We conclude that ESA is a sensitive and convenient method to directly screen for ESBL-producing organisms in clinical specimens.

P872 Detection of ESBLs in various *Enterobacter cloacae* isolates in the Polish External Assurance National Quality Control Scheme POLMICRO 2007

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Objectives: The aim of this study was to evaluate quality of performance of microbiology diagnostic laboratories in Poland. POLMICRO is the National External Quality Assurance Scheme Programme in Poland. It addresses several important questions in bacterial identification and susceptibility testing. In one of 2007 editions *Enterobacter cloacae* was selected for distribution as a resistant alert pathogen.
Methods: Nine isolates of *E. cloacae* were prepared as a part of POLMICRO 2007/III. Each of 476 laboratories participating in the programme received a set of three phenotypically different isolates. The aim of every laboratory was to identify the strains to the species level, provide susceptibility testing results and to detect and interpret the AmpC- and/or ESBL-mediated resistance. The results were delivered via Internet. The study set of *E. cloacae* strains included: a) strains PM-107, PM-108 and PM-109 with AmpC derepression, b) strains PM-110, PM-111 and PM-112 with inducible AmpC and ESBL, and c) PM-115, PM-116, PM-117 with AmpC derepression and ESBL.
Results: Results were received from 467 (99.36%) laboratories and 434 laboratories obtained acceptable results. Because of the educational character of the control some kinds of errors were permitted. Four-hundred and sixty-four laboratories correctly identified isolates to the species level. Most of laboratories correctly determined susceptibility to given antibiotics and provided correct clinical interpretation. The minority of laboratories had difficulties with the proper interpretation of resistance mechanism. Some of these, 9.66% have misinterpreted the AmpC derepressed mutants as ESBL producers. Some others, 3.65% have not detected ESBLs in wild-type strains whereas 13.09% laboratories have not identified ESBLs in the context of the AmpC derepression.
Conclusions: The great majority of participating laboratories (93%) were able to properly detect β-lactamase mediated resistance mechanisms in *E. cloacae*. The results achieved by most laboratories in POLMICRO 2007/III indicated their competence in the area covered by the control.

P873 Rapid Etest MIC testing using CHROMagar and Mueller-Hinton agar for Gram-positive and Gram-negative aerobes

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Objectives: Same day MIC results of key antibiotics against important Gram+ and Gram− pathogens can be clinically valuable. Rapid results can be used to verify, correct, adjust or de-escalate empiric therapy, as necessary. Appropriate and adequate therapy implemented early can impact clinical outcome and save significant costs. We compared a

prototype CHROMagar (CMA) to Mueller Hinton agar (MHA) for MIC testing with Etest to evaluate the possibility of obtaining same day results for 12 antibiotics of choice for 14 clinically important species of aerobes.
Methods: 108 challenge strains, including MRSA, GISA/hGISA, VRE, ESBL, AmpC, MBL, OXA phenotypes used comprised: staphylococci (18), enterococci (11), *Escherichia coli* (17), *Klebsiella pneumoniae* (6), *Enterobacter cloacae* (5), *Citrobacter freundii* (5), *Serratia marcescens* (5), *Pseudomonas aeruginosa* (16), *Acinetobacter baumannii* (10), *Stenotrophomonas maltophilia* (10) and *Burkholderia cepacia* (5) and 9 ATCC reference strains. Etest strips (Gram+: oxacillin, vancomycin, linezolid, daptomycin, tigecycline, gentamicin, tetracycline, ciprofloxacin, rifampicin, clindamycin, erythromycin, trim/sulfa), (Gram-: ciprofloxacin, amikacin, tobramycin, gentamicin, aztreonam, piperacillin/tazobactam, cefepime, cefotaxime, imipenem, tigecycline, colistin, trim/sulfa) were tested on CMA and MHA according the manufacturer's instructions and read at 4, 6, and 24 h.
Results: Good MIC agreement between CMA and MHA (80–100% +/− 1 dilution) was seen at 24 h for 153/168 (91%) of drug/species combinations (>8000 data points). Enterobacteriaceae could be read at 6 h and results agreed (+/-1 dilution) with 24 h values for > 90% of cases for both media. 50% of Gram+ strains could be read at 6 h while Gram-negative non fermenters (GNF) and certain resistant phenotypes could only be read after overnight incubation. Colour changes on CMA were useful species markers.
Conclusion: Rapid same day Etest MIC results for key antibiotics could be reliably obtained for Enterobacteriaceae, and some staphylococci and enterococci with CMA and MHA. GNF and certain resistant phenotypes required overnight incubation. CMA should be further evaluated for specimen MIC testing to generate same day results for urgent clinical cases.

P874 Evaluation of a new Etest strip for detection of plasmidic AmpC

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Objectives: Simple and reliable methods for detection of plasmid mediated AmpC phenotypes are needed in clinical laboratories. In a previous study (ICAAC abstract D-0451, 2006), Etest prototype strips for detection of AmpC based on cefotetan (CN) ± cloxacillin (CLO) was found to be useful. However, cefotetan powder is no longer readily available. Cefoxitin (FX) resistance used as a screen for AmpC, comprise an alternative to CN for the design of a FX based test. This new FX±CLO prototype strip was compared to CN±CLO using genotypically characterised strains.
Methods: FX 512–8 ug/mL ±CLO double-sided Etest strip was tested with 48 clinical strains comprising: *Escherichia coli* (24), *Klebsiella pneumoniae* (7), *Proteus mirabilis* (1), *Serratia marcescens* (4), *Klebsiella oxytoca* (1), *Enterobacter aerogenes* (7), *Enterobacter cloacae* (2), *Morganella morganii* (2). AmpC genotypes (PCR based) included CMY-1, CMY-2, DHA-1, ACC, FOX and MIR. Standard Etest MIC procedure for Gram-negative aerobes was used and reduction of the cephamycin MIC by ⩾3 dilutions by CLO or the presence of a "phantom zone" or deformation of inhibition ellipses after 16–20 h incubation was interpreted AmpC positive.
Results: See table.

		Etest AmpC Results			
		CN±CLO		FX±CLO	
Phenotype	Strains (N)	+	−	+	−
Plasmidic AmpC	29	29	0	26	3
Chromosomal AmpC	15	13	2	10	5
Negative controls	4	0	4	0	4
	Detection of Plasmidic AmpC (%)				
	Sensitivity	100		90	
	Specificity	100		91	

Conclusion: Etest FX±CLO showed acceptable sensitivity (90%) and specificity (91%) for detection of various genotype plasmidic AmpC enzymes and may comprise an alternative diagnostic tool for routine use in clinical laboratories.

P875 Detection of plasmid mediated AmpC β-lactamase in clinical isolates by three different methods

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Objectives: AmpC mediated β-lactam resistance in *Escherichia coli* and *Klebsiella* spp. is an emerging problem all over the world. High level AmpC production is typically associated with in vitro resistance to all β-lactams except for carbapenems and cefepime. AmpC detection is important to ensure effective therapeutic intervention and optimal clinical outcome. Nevertheless, detection of AmpC mediated β-lactam resistance is a challenge for laboratories. There is no recommended CLSI guidelines for detection of this resistance mechanism. The aim of our study is to address this issue by tetsting different methods.
Methods: A total of 210 blood isolates of *E. coli* and *Klebsiella* spp. resistant to cefoxitin, amoxycillin-clavulanic acid, aztreonam, and any of cephalosporins were taken to the study. All isolates were identified by Phoenix automatised system. Susceptibility testing against imipenem, ceftazidime, cefotaxime, aztreonam, cefepim, cefotetan, cefpodoxime, cefoxitin, amikacin, tobramycin, amoxicillin-clavulanic acid and ciprofloxacin were done by disc diffusion. For the phenotypic detection of plasmid mediated AmpC β-lactamase inhibitor based method with boronic acid and modified-three dimensional test were used. The isolates found as AmpC β-lactamase positive based on phenotypic results, were confirmed by multiplex PCR for the presence of six different classes of AmpC β-lactamase coding genes.
Results: Boronic acid inhibition test revealed 46 positive isolates. When the inhibition test performed by boronic acid was coupled with clavulanic acid to rule out ESBL presence, the number of positive isolates decreased to 20. Modified-three dimensional test yielded 26 positive results. With multiplex PCR 4 isolates were confirmed as plasmid mediated AmpC β-lactamase producers. All of these four isolates had inducible type AmpC β-lactamase and none of them had ESBL.
Conclusion: Although boronic acid inhibition test can be useful for phenotypic detection of AmpC type β-lactamase, clavulanic acid must be added to the inhibition test in order to prevent the false positive results on account of presence of ESBL. For the confirmation of presence of AmpC β-lactamase, molecular methods, although expensive and labour intensive, should be used in clinical laboratories.

P876 Phenotypic AmpC detection – which inhibitor is best?

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Objectives: Synergy testing for conformation of AmpC activity is not so well established as for ESBLs, and the inference of AmpC phenotypes often relies on indirect indicators, e.g. resistance to cefotaxime and cefoxitin but not cefepime. Oxacillins and boronic acid derivatives, do however, inhibit AmpC enzymes and we compared their utility in synergy based detection tests for these enzymes.
Methods: The potential inhibitors, cloxacillin 100 mg/L, benzo(b)thiophene-2-boronic acid 100 mg/L and phenylboronic acid 200 mg/L were tested in combination with cefotaxime, ceftazidime, cefpirome and cefoxitin by the BSAC agar dilution method against 71 oxyimino-cephalosporin or carbapenem-resistant isolates with a variety of known resistance mechanisms.
Results: In general, cefotaxime was the best indicator substrate for AmpC hyperproduction in Enterobacteriaceae, irrespective of the inhibitor. Thus, with either boronic acid derivative ⩾8 fold synergy was seen with cefotaxime for >90% of AmpC hyperproducing Enterobacteriaceae, with fewer than 5% false positive results for strains with ESBLs or other resistance mechanisms. Cloxacillin gave ⩾4-fold synergy for ⩾90% of AmpC producing Enterobacteriaceae too but this low potentiation threshold was associated with poor specificity, with 30%

false positives among the isolates with other modes of cephalosporin resistance. Moreover cloxacillin failed to potentiate cephalosporin MICs for AmpC-hyperproducing *P. aeruginosa* isolates; whereas 16-fold, synergy typically was seen for the boronic acid, ceftazidime combinations. Some failures (mostly with *Enterobacter*) occurred when ESBLs were present together with AmpC enzymes.
Conclusion: Benzo(b)thiophene-2-boronic acid/cefotaxime combinations provided the most effective AmpC synergy test for Enterobacteriaceae, with cefotaxime MICs potentiated ⩾8 fold for >90% of the AmpC producers and with good specificity. Boronic acid/ceftazidime combinations were more suitable for *P. aeruginosa*.

P877 Evaluation of Sensititre® Yeastone® for direct antifungal susceptibility testing from blood cultures

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Objectives: The aim of this study was to evaluate Sensititre YeastOne (TREK Diagnostic Systems, Cleveland, USA), already used in our routine diagnostics for performing in-vitro antifungal susceptibility testing, starting directly from the bottle of blood culture positive for yeasts, with the explicit aim of intervening to reduce the TAT for antifungal susceptibility testing.

Organisms (no. tested) and antifungal agents	MIC (ug/ml)					
	Range		MIC_{50}		MIC_{90}	
	Reference procedure	Direct procedure	Reference procedure	Direct procedure	Reference procedure	Direct procedure
Candida albicans (20)						
amphotericin	0.008–0.06	0.008–0.06	0.03	0.03	0.06	0.06
fluconazole	0.125–0.25	0.125–0.25	0.125	0.125	0.25	0.25
itraconazole	0.016–0.03	0.016–0.03	0.016	0.016	0.03	0.03
ketoconazole	0.008–0.016	0.008–0.016	0.008	0.008	0.016	0.016
5-fluorocytosine	0.03–0.06	0.03–0.06	0.03	0.03	0.06	0.06
voriconazole	0.008–0.016	0.008–0.016	0.008	0.008	0.016	0.016
Candida glabrata (10)						
amphotericin	0.03–0.25	0.03–0.25	0.06	0.06	0.125	0.125
fluconazole	16–32	16–32	16	16	16	16
itraconazole	0.5–4	0.5–4	1	1	2	2
ketoconazole	0.016–2	0.016–2	1	1	2	2
5-fluorocytosine	0.03–2	0.03–2	0.06	0.06	0.5	0.5
voriconazole	0.25–2	0.25–2	0.5	0.5	1	1
Candida tropicalis (4)						
amphotericin	0.25–1	0.25–1	0.25	0.25	0.5	0.5
fluconazole	2–8	2–8	4	4	8	8
itraconazole	0.25–2	0.25–2	0.5	0.5	1	1
ketoconazole	0.125–1	0.125–1	0.5	0.5	1	1
5-fluorocytosine	0.25–4	0.25–4	0.5	0.5	2	2
voriconazole	0.008–1	0.008–1	0.06	0.06	0.5	0.5
Candida parapsilosis (4)						
amphotericin	0.125–1	0.125–1	0.25	0.25	0.5	0.5
fluconazole	2–8	2–8	4	4	8	8
itraconazole	0.125–1	0.125–1	0.25	0.25	0.5	0.5
ketoconazole	0.125–1	0.125–1	0.25	0.25	0.5	0.5
5-fluorocytosine	0.125–1	0.125–1	0.25	0.25	1	1
voriconazole	0.016–0.5	0.016–0.5	0.06	0.06	0.25	0.25
Candida krusei (2)						
amphotericin	0.25–1	0.25–1	0.25	0.25	0.5	0.5
fluconazole	128–256	128–256	256	128	256	128
itraconazole	0.5–2	0.5–2	1	1	2	2
ketoconazole	0.5–2	0.5–2	1	1	2	2
5-fluorocytosine	0.125–16	0.125–16	0.5	0.5	4	4
voriconazole	0.125–2	0.125–2	0.25	0.25	1	1

Methods: In our laboratory, blood culture bottles are normally incubated using the system BacT/ALERT 3D (bioMerieux, Marcy l'Etoile, France). Sensititre YeastOne was used by direct inoculation from positive blood culture bottles in 40 cases of candidaemia. All the results obtained directly from a positive bottle were compared with those obtained using standard laboratory procedures, after sub-culturing from a positive bottle onto solid media. The strain of *Candida parapsilosis* ATCC 22019 was used for quality control. The direct susceptibility test was performed as follows: the positive blood culture bottle was shaken and used to prepare a Gram stain to confirm the presence of yeast monomicrobism. Five to six ml of the sample were aspirated with a vacutainer test tube (Sarstedt, Nümbrecht, Germany) and centrifuged at 3200 revs for 15 minutes; the microbial pellet was gradually suspended in a small aliquot of physiological solution then diluted with physiological solution in order to obtain turbidity of 0.5 McFarland. This final suspension was used to perform the susceptibility test according to the manufacturer's instructions. Incubation at 35°C was continued for 24 hours.
Results: The table summarises the in vitro susceptibilities (MIC's range, MIC 50 and MIC 90) of the 40 isolates of *Candida* spp., compared with the results obtained in accordance with the standard procedures. Considered as a whole, out of a total of 40 strains tested, no very major errors or major errors were revealed and only five minor errors (98% agreement rate out of a total of 240 drug/bug combinations tested).
Conclusion: The results obtained from direct inoculation of Sensititre YeastOne demonstrated excellent performance and compared very well with tests performed using standard procedures. In addition, results were easy to interpret and available on average 3 days earlier than conventional results, allowing a potentially very significant reduction in the TAT and providing tangible information, about the current management of antifungal therapy or, if necessary, its modification.

P878 Detection and reporting betalactam resistance phenotypes in *Escherichia coli* and *Klebsiella pneumoniae*: a multicentre proficiency study in Spanish laboratories

M.C. Conejo, C. Mata, F. Navarro, Á. Pascual and the GEMARA collaborative group

Objective: To evaluate the ability of Spanish microbiology laboratories in detecting and reporting betalactam resistance phenotypes in well-characterised clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* expressing extended-spectrum β-lactamases (ESBL) or AmpC β-lactamases.
Methods: Twelve well-characterised strains were sent to 57 laboratories: 6 clinical isolates expressing the most prevalent ESBL in our country (2 CTX-M-10, 1 CTX-M-9, 1 CTX-M-14, 1 TEM-4 and 1 SHV-12), 3 isolates producing AmpC-type enzymes [ACBL; 2 plasmid-mediated (FOX-5 and CMY-2) and one *E. coli* hyperproducing chromosomal ACBL], and 3 ATCC reference strains (1 with SHV-8). Laboratories were requested to use routine methods for identification and antimicrobial susceptibility testing (AST), considering the strains as blood isolates. Reference results were established by two independent laboratories by using API strips for identification and CLSI microdilution and disc diffusion methods and interpretative criteria for AST.
Results: Automated systems were used by 61% of laboratories for bacterial identification and AST. The remaining laboratories used semi-automated or manual systems. Ninety one percent of laboratories correctly recognised all seven ESBL producers and consequently low rates of errors were observed when testing cephalosporins and aztreonam. The highest rates of errors with all the ESBL producers were observed when testing the penicillin/β-lactamase inhibitor combinations, but in more than 60% of cases these errors were not related to the MIC value but to the microbiologist interpretation (minor errors). No discrepancies were observed for carbapenems in ESBL producers. Correct recognition of all three ACBL producers was assessed by 47% laboratories. ACBL producers were erroneously reported as ESBL producers in 11 cases and as penicillinase hyperproducers associated or not to permeability reduction in 10 cases. Interpretative criteria applied to ACBL phenotype were not uniform, but a tendency of considering carbapenems as the only therapeutic option was observed.
Conclusions: Most laboratories in Spain detected the ESBL phenotype, but detection of the ACBL phenotype by routine methods needs to be improved.

P879 Assessment of capacity of Russian microbiological laboratories to detect ESBL production in Gram-negative bacteria by phenotypic methods

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Backgound: Patients with infections caused by ESBL-producing organisms are at an increased risk of treatment failure with β-lactam antibiotics. Thus ability of microbiological laboratory to detect ESBLs is very important. No adequate information of ability of Russian microbiological laboratories to detect ESBL is available so far.
Objectives: To assess capability of microbiological laboratories in different regions of Russia to detect ESBL production in Gram-negative bacteria with phenotypic methods.
Methods: This study was a part of nationwide external quality control program. Forty three microbiological laboratories from 22 regions of Russia participated in the study. Two "blinded" strains (ESBL-producing strains of *Enterobacter cloacae* and *Providencia rettgeri*) were distributed for identification, susceptibility testing to cefotaxime (CTX), ceftazidime (CAZ), cefepime (CFP) and interpreting resistance phenotypes (including ESBL) with any test routinely used in the laboratory. E.cloaceae was producing TEM-29 (ESBL). *P. rettgeri* was producing TEM-1 and STX-M-3 (ESBL). MICs of both strains for different antimicrobials are presented in the Table. Sises of zones of inhibition for CTX, CAZ and CFP formally were in “susceptible” range for both strains according to CLSI breakpoints.
Results: In 3 of 43 laboratories automatic susceptibility testing systems (ATB Expression and VITEK) were used, in remaining 40 laboratories susceptibility testing has been performed by disk diffusion method. In all laboratories identification was done correctly. ESBL production in E.cloaceae was detected only in 4 (9.3%) laboratories and the strain was considered as resistant to CTX, CAZ and CFP. None of these laboratories used automatic susceptibility testing systems (ASTS). In all laboratories using ASTS E.cloaceae strain has been reported as susceptible to CTX, CAZ and CFP. ESBL production in *P. rettgeri* was detected in 7 (16.3%) laboratories, 3 of which used ASTS and the strain was considered as resistant to CTX, CAZ and CFP.

Table: MICs of tested strains for antimicrobials (mg/L)

Strain	CTX	CTX/CL	CAZ	CAZ/CL	CFP	CFX	AMX	AMX/CL
E. cloacae	0.25	0.25	8	0.25	1	64	256	64
P. rettgeri	8	0.125	0.5	0.25	8	2	256	256

CTX = cefotaxime, CAZ = ceftazidime, CFP = cefepime, CFX = cefoxitine, CL = clavulanic acid, AMX = amoxicillin.

Conclusion: Capacity of routine clinical microbiological laboratories in Russia to detect ESBL production in Gram-negative bacteria is inadequate and needs to be improved.

β-lactamases: laboratory detection

P880 Validation of the extended-spectrum β-lactamase phenotype with molecular methods

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Objectives: In order to validate extended-spectrum β-lactamase (ESBL) screening and confirmation assays, we determined the presence of 6 different ESBL-encoding genes in a representative collection of Swiss isolates showing an ESBL phenotype. In the subset of isolates in which no ESBL genes were detected, the presence of SHV-1 hyperproducing penicillinase, group D oxacillinases, and plasmid-mediated AmpC β-lactamases was additionally assessed.
Methods: The clinical Enterobacteriaceae isolates expressing an ESBL phenotype were consecutively collected at the University Hospital Basel (n = 39) or obtained from several Swiss laboratories (n = 33). As phenotypic confirmatory test, three different ESBL Etest strips (AB Biodisk, Sweden) were used: ceftazidime, cefotaxime, and cefepime, each plus/minus clavulanic acid. Repetitive patient isolates and K1 hyperproducing *Klebsiella oxytoca* were excluded. To determine the molecular types, PCR amplifying SHV, TEM, CTX-M, VEB, PER, GES, OXA-1, OXA-10 genes as well as genes of the SHV-1 promoter region and of 6 different phylogenetic groups of AmpC was performed and amplicons were sequenced.
Results: Among 162 isolates showing an ESBL phenotype, molecular analysis demonstrated that 103 (63.6%) expressed a CTX-M type, 23 (14.2%) a SHV ESBL-type, 17 (10.5%) a TEM ESBL type, one GES-1, and one PER-1 ESBL. Among the 21 (13.0%) isolates in which no ESBL-genes could be detected, 12 *Klebsiella pneumoniae* and 6 *Escherichia coli* harboured the typical C to A mutation in the second position of the SHV-1 –10 promoter region associated with hyperproduction of SHV-1. These 18 isolates showed a characteristic susceptibility and ESBL screening/confirmation pattern that differed significantly from all molecularly confirmed ESBL isolates except for GES-1. The remaining two *E. coli* and one *Citrobacter koseri/ amalonaticus* contained an OXA-1 gene explaining the false positive cefepime/cefepime + clavulanic acid Etest.
Conclusions: Almost all phenotypic ESBL isolates in which no ESBL genes could be detected were SHV-1 hyperproducers known leading to false positive results in phenotypic ESBL tests. The relatively high number of false positive results might be explained by the low incidence of ESBLs in Switzerland. Because of their unique characteristics determined in this study, SHV-1 hyperproducers can be excluded.

P881 Detection of ESBL production by the Hyplex® ESBL ID multiplex PCR-ELISA system

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Objectives: Extended-spectrum β-lactamases (ESBL), the enzymes that are able to hydrolyse diverse β-lactam antibiotics, are widely distributed among Enterobacteriaceae. This study was performed in order to compare a novel multiplex-PCR-ELISA-System with the conventional microbiological methods for the detection of ESBL-producing blood isolates of *Klebsiella pneumoniae*, in a Greek tertiary hospital.
Methods: Sixty-two different *K. pneumoniae* isolates from blood cultures of inpatients (77% ICU, 18% medical wards, 5% surgical wards) were collected over a two year period. Two *Escherichia coli* blood isolates one positive and the other negative for ESBL production, were used as positive and negative controls respectively. They were identified by standard methods and MICs were determined by the VITEK 2 automated system, according to CLSI guidelines. ESBL production was screened by Etest ESBL. The Hyplex® ESBL ID method involves amplification of blaTEM, blaSHV, blaCTX-M and blaOXA bacterial DNA by multiplex PCR and hybridisation of the PCR products to specific oligonucleotide probes: a Consensus probe (TEM, SHV, CTX-M and OXA specific) and a CTX-M specific probe. Nine of the above positive blood cultures were tested directly with the Hyplex® ESBL ID test.
Results: Sixty-one out of sixty-two *K. pneumoniae* blood isolates were found to produce an ESBL activity by the Etest. The same isolates gave a positive result with the Consensus probes demonstrating the presence of ESBL phenotype-associated genes (sensitivity 100%, specificity 100%). Only three of the sixty-one isolates gave an additional positive result with the CTX-specific probes proving ESBL production. The nine blood cultures that were tested directly gave identical results with their respective isolates.
Conclusion: The hyplex®ESBL ID test can be reliably applied for the detection of ESBL genes carriage by Gram-negative species directly in positive blood cultures or in blood isolates. This diagnostic utility may be very useful for the timely detection of ESBL genes and has to be evaluated.

P882 ChromID ESBL agar for the detection of extended-spectrum β-lactamase producing Enterobacteriaceae

J. Montgomery, J. Wang, J. Nakos, V. Gurtler (Melbourne, AU)

Objectives: Extended spectrum β-lactamases (ESBL) are major nosocomial pathogens, and more recently have emerged in community patients. This study evaluated chromID ESBL (chromID) chromogenic agar for the rapid detection of ESBL in Enterobacteria as part of the routine identification and susceptibility testing of isolates, and was compared with molecular detection.
Method: Using a multipoint inoculator (in-house agar dilution method) 244 pure cultures of Enterobacteria was stamped onto chromID agar, as part of the daily identification procedure. In addition Jarlier's double disk synergy tests were also performed. PCR for CTX-M, SHV and TEM genes was performed on all isolates. AmpC PCR was also done.
Results: Of 244 isolates, 97 were ESBL positive by PCR. These were *Citrobacter freundii* (6), *Enterobacter aerogenes* (5), *Enterobacter cloacae* (30), *Escherichia coli* (30), *Klebsiella oxytoca* (10), *Klebsiella pneumoniae* (13), *Proteus penneri* (1) and *Proteus vulgaris* (2). Enzymes detected included TEM (45), SHV (42) and CTX-M (65). Multiple genes were detected in 51 (53%) of isolates. Five PCR positive isolates that failed to grow on chromID, were *K. pneumoniae* (CTX-M, TEM and SHV), *K. oxytoca* (CTX-M), *E. aerogenes* (SHV) and two *E. coli* one with SHV and the other TEM. There were 36 that grew on chromID but were not confirmed by PCR. Of these false positives 27 (75%) were chromosomal or plasmid-mediated AmpC producers. Of 30 Enterobacteria that grew on chromID but produced no pigment, 13 were ESBL producers and included *C. freundii* (3), *E. coli* (6), *K. oxytoca* (1), *E. cloacae* (2) and *P. vulgaris* (1). The sensitivity and specificity of chromID was 95% and 80%.In comparison Jarlier's disk synergy testing were positive in 64 isolates of which 58 were ESBL producers. This method did not detect 39 of the ESBL producers. Sensitivity was 60% and specificity of 96%.
Conclusions: ChromID agar is a very sensitive medium for the detection of ESBL-producing Enterobacteria. Some isolates that grow on the medium but have no pigmentation are ESBL producers. AmpC producing Enterobacteria are a significant source of false positive growth.

P883 Comparison of phenotypic tests for detection of plasmid-mediated AmpC β-lactamases

J. Montgomery, J. Nakos, V. Gurtler (Melbourne, AU)

Objectives: Detection of plasmid-mediated AmpC β-lactamases is important for optimal outcome in patients with serious infections. Laboratory detection of these enzymes is difficult. This study compared six phenotypic methods of detection of plasmid-mediated AmpC β-lactamases with PCR.
Methods: Organisms selected included 106 *Escherichia coli*, 17 *Klebsiella* spp., three *Proteus mirabilis* and two *Salmonella* spp. Etest MICs wre performed on all isolates. Multiplex PCR comprised MOX, CMY, DHA, ACC, EBC and FOX genes. Phenotypic tests; cefoxitin resistance (CR): disk testing with benzeneboronic acid (BB), benzothiopene-2-boronic acid (BT) and 3-aminophenyl boronic acid (AB) each combined with a third generation cephalosporin with and without the boronic acid derivative, (a zone diameter difference of five millimetres or more indicated enzyme presence): tris-EDTA (TE) and cefoxitin with a flattening of the zone indicating inhibition; Cica-beta-C-test (CC) using the chromogenic cephalosporin HMRZ-86, combined with a boronic acid inhibitor. Disk diffusion tests used standard CLSI methods. Etest MIC were performed according to the manufacturers instructions and interpreted according to CLSI criteria.
Results: Of a total of 128 isolates studied 29 were PCR positive. Twenty-one of 106 *Escherichia coli*; five of 15 *Klebsiella pneumoniae*, one of two *Klebsiella oxytoca* and two *Salmonella* spp. were PCR positive. Enzymes detected were DHA (13) and CIT (16). Three *Proteus mirabilis* were PCR negative. Six PCR positive and 57 PCR negative isolates had cefoxitin Etest MIC results equal to or less than 16 mg/L. The table summaries the results when compared with AmpC detection by PCR.

Phenotypic method	Sensitivity %	Specificity %	Positive Predictive Value	Negative Predictive Value
CR	76	58	34	89
BB	76	80	52	92
BT	72	61	35	88
AB	69	88	63	91
TE	45	92	62	85
CC	31	91	53	82

Conclusions: No one phenotypic method tested reliably detected all plasmid-mediated AmpC enzymes. A combination of CR and CC may be useful but requires further study. Screening methods still require PCR confirmation.

P884 Development and validation of a novel media (CHROMagar ECC CTX-M) for isolation of CTX-M producing Enterobacteriacae whilst inhibiting ampC producing organisms

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Objectives: To develop and validate a novel media based on CHROMagar ECC to isolate bacteria containing the blaCTX-M gene whilst inhibiting ampC strains and to evaluate different phenotypic tests for identifying blaCTX-M strains as being ESBL producers.
Methods: A panel of strains was assembled to assess different agars for ability to isolate bacteria containing the blaCTX-M gene whilst inhibiting ampC strains. The panel comprised 123 isolates (mainly *E. coli*) and was characterised genotypically (tested for blaCTX-M blaOXA, blaSHV, blaTEM and ampC genes) and phenotypically (cefepime, cefotaxime, cefpodoxime and ceftazidime MAST EsbL ID disks, the Cica-Beta test and MICs). The final evaluation panel comprised ~ 54% and ~ 32% of strains positive for blaCTX-M and ampC genes respectively. A range of agents was evaluated as additives to CHROMagar ECC and the most optimal combination selected to produce CHROMagar ECC CTX-M. The CHROMagar ECC CTX-M was assessed against other agars with and without enrichment for the isolation of blaCTX-M positive organism from bovine faeces spiked with 10^1 to 10^4 cfu/g of blaCTX-M strains with and without the presence of ampC strains.

Table 1: Recovery of CTX-M positive colonies from bovine faecal samples spiked with ~10^1 to 10^4 cfu/g CTX-M positive strains with and without ~10^4 cfu/g ampC positive strains

Agar	% of colonies that were CTX-M positive for spike levels; **With/without presence of ampC strains**			
Spike level	**10^1**	**10^2**	**10^3**	**10^4**
CA-ECC	0/0	17/0	0/0	20/17
CA-ECC + 2 μg/ml CTX	0/80	0/17	50/100	50/100
CA-ECC + 8 μg/ml CTX	17/100	0/100	17/100	50/100
bioMérieux ESBL	0/67	0/50	33/83	83/100
CA-EEC-CTX-M	**67/83**	**67/100**	**67/100**	**67/100**

CA-ECC, CHROMagar ECC; CA-EEC-CTX-M, CHROMagar ECC CTX-M, CTX, cefotaxime. If no colonies were recovered from faeces directly cultured onto agars, then enrichment was used (faeces overnight in buffered peptone water at 37º).

Results: CHROMagar ECC CTX-M showed a 98.5% sensitivity and 83.6% specificity for growth of the blaCTX-M positive strains in the panel and this was superior to CHROMagar ECC with addition of different concentrations of either cefotaxime or ceftazidime and to bioMérieux ESBL-Bx agar. CHROMagar ECC CTX-M was also overall superior to other agars for the isolation of blaCTX-M strains from bovine

faeces, and was capable at detecting ~ 10 cfu blaCTX-M strains per gram of faeces, even in the presence of ampC strains (Table 1). The sensitivity and specificity of MAST ESbL ID disks for confirming the blaCTX-M strains as ESBL strains ranged from 93.9% to 100% for the each set of disks but the sensitivity and specificity could be increased to 98.5% and 100% respectively by taking results from two sets of disks. The Cica-Beta test showed a 77.3% and 90.9% sensitivity and specificity for confirming the blaCTX-M strains as ESBL strains.
Conclusion: The results show that CHROMagar ECC CTX-M is a highly sensitive, selective and specific agar for isolating medically important blaCTX-M strains, particularly in the presence of ampC strains. The MAST ESbL ID disks proved to be highly sensitive and specific at confirming blaCTX-M strains as ESBL's, particularly if results were taken from two sets of disks.

P885 Evaluation of a chromogenic medium (chromID™ ESBL, BioMérieux) for the screening of ESBL-producing *Escherichia coli*

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Introduction: Detection of Extended-Spectrum-Beta-Lactamase (ESBL) producing *E. coli* isolates relies on decreased susceptibility for at least one of the indicator antibiotics as recommend by the CLSI. The results are subsequently confirmed in an ESBL confirmation test. This two-step procedure is necessary to increase the specificity as decreased susceptibility may rely on mechanisms other than ESBL. Since ESBLs are increasing worldwide companies have developed selective chromogenic media in which the most important ESBL-producing organisms are identified by a difference in colony colours. Growth followed by colourisation is only possible if organisms possess β-lactamases capable of hydrolyzing the indicator antibiotics present in the agar.
Objectives: to evaluate the performance of a chromogenic medium (chromID tm ESBL, BioMérieux) compared to the CLSI ESBL-screening procedure.
Methods: 1968 *E. coli* strains prospectively sampled between 1/9/2006 and 1/9/2007 isolated from community and hospitalised patients were included. Susceptibility testing is performed by agar dilution and ESBL detection is performed using ceftazidime, ceftriaxone and aztreonam as indicator antibiotics. *E. coli* strains with MICs > 1 mg/l for either agent are subjected for confirmation. Confirmation for ESBL is performed by combined double disks (DD) of Becton Dickinson (Sparks, MD, USA), consisiting of cefotaxime (30 micg) or ceftazidime (30 micg) alone and combined with clavulanic acid (10 micg). All isolates growing on the chromID tm ESBL with pink to burgundy colours were also subjected to the same confirmation procedure.
Results: 1799 isolates were ESBL negative according to the CLSI guidelines and did not grow on ChromID tm ESBL plates. 169 isolates were tested positive of which 81 also grew on ChromID tm ESBL plates. 75 out of the 81 were confirmed ESBL-positive by DD. 64 isolates out of 88 not growing on ChromID tm ESBL but positive in the CLSI screening were negative in the DD confirmation. One isolate missed by ChromID tm was tested positive in the DD confirmation. 23 remaining isolates were not tested.

	ChromID ESBL pos	ChromID ESBL neg	
CLSI pos	81	88	169
CLSI neg	0	1799	1799
			1968

Conclusion: Application of the CLSI guidelines resulted in twice as much isolates suspected for ESBL as by ChromID tm ESBL-agar plates. However, the majority of the extra positives are false positive probably due to the presence of other resistance mechanisms. Screening for ESBL with ChromID tm ESBL-agar plates has a higher specificity than the method advocated by CLSI.

P886 Evaluation of a chromogenic agar plate as screening medium for the detection of extended-spectrum β-lactamase-producing Enterobacteriaceae

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Objectives: To evaluate the performance of a selective chromogenic agar medium (ChromID ESBL, bioMérieux) as primary medium for the detection of extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae directly from clinical specimens.
Methods: Clinical specimens (urine samples, lower respiratory tract samples, perineal swabs and wound swabs) were prospectively collected from a hospitalised patient population. Specimens were inoculated on the ESBL medium and the in house media (MacConkey agar medium and blood agar medium). Isolates were identified to the species level by use of VITEK 2 ID cards (bioMérieux). Isolates were screened for ESBL production by MIC analysis and the ESBL test with VITEK 2 AST-N045 cards (bioMérieux). ESBL production was confirmed by use of the combined-disk method (ceftazidime and cefepime with and without clavulanic acid, Rosco) and the E-test method (cefotaxime, ceftazidime and cefepime with and without clavulanic acid, AB Biodisk).
Results: A total of 558 specimens were obtained from 374 patients. The ESBL medium yielded growth of 111 isolates from 101 specimens (18%). These 101 isolates consisted of 57 Enterobacteriaceae isolates (51%). The non-Enterobacteriaceae isolates belonged mainly to the species *Pseudomonas aeruginosa*. Of the 57 Enterobacteriaceae isolates, 30 isolates were confirmed as being ESBL producers (specificity 27%). The non-ESBL-producing Enterobacteriaceae isolates consisted mainly of the genus *Enterobacter*. A total of 31 ESBL-producing Enterobacteriaceae were isolated in this study. All but one of these isolates were recovered from the ESBL medium (sensitivity 97%).
Conclusion: The chromogenic ESBL medium is a sensitive medium for the detection of ESBL-producing Enterobacteriaceae. Regarding the specificity, the growth of non-fermenting Gram-negative rods is a frequent finding. However, use of the cytochrome oxidase test enables rapid identification of most of these bacteria. A more important issue is the growth of Enterobacteriaceae with other resistance mechanisms then ESBL production. This makes the use of confirmatory testing obligatory for species known to harbour these non-ESBL resistance mechanisms.

P887 Comparative performance of three phenotypic confirmatory Etest strips for the detection of extended-spectrum β-lactamases-producing Enterobacteriaceae in Belgian hospitals

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Objectives: Criteria for ESBL detection have only been established for a limited number of Enterobacteriaceae species (*E. coli*, *Klebsiella* spp., *Proteus mirabilis*) by the Clinical Laboratory Standards Institute (CLSI). ESBL production in other species is clinically significant, but remains under reported owing to the lack of consensus guidelines. We evaluated the performance of 3 phenotypic confirmatory Etest strips to detect ESBL producers among a large collection of Enterobacteriaceae clinical isolates collected in Belgian hospitals.
Methods: 332 ESBL-producing Enterobacteriaceae clinical isolates (149 *E. aerogenes*, 131 *E. coli*, 31 *K. pneumoniae* 11 *E. cloacae*) obtained in 2006 from 86 centres were investigated. ESBL production was assessed by multiplex PCR analysis (TEM, SHV, CTX-M) and DNA sequencing. Three Etest strips containing cefotaxime (TX), ceftazidime (TZ), and cefepime (PM) alone and in combination with clavulanate (CL) were evaluated. Etest results were considered positive according to the CLSI criteria established for *E. coli*, *Klebsiella* spp. and *P. mirabilis* (i.e., a > 3 two-fold concentration decrease in MIC tested in combination with clavulanate versus its MIC when tested alone) or when a deformation

of the inhibition ellipse was observed. Indeterminate Etest results were considered negative.

Results: Isolates were found to possess blaTEM (n = 300; 153 with TEM-24 type) blaSHV (n = 31) with SHV-4, -5, and -12 as the most common types) and CTX-M (n = 35; CTX-M-15, M-1, M-9, M-14, M-2 as the most frequent). Ability of Etest strips in detecting ESBL phenotypes in all species isolates was as follows: PM/PM-CL (95.8%), TZ/TZ-CL (54.8%), and TX/TX-CL (53.3%). Against *Enterobacter aerogenes* and other AmpC-positive species (n = 365), accurate ESBL detection was consistently achieved only by PM/PM-CL (89.7%; 93.2% in *E. aerogenes*) while Etest results were mostly indeterminate due to off scale values for TX and TZ-based Etests. Against non-AmpC producing species (n = 367), sensitivity of ESBL detection was: TZ/TZ-CL (92.8%), TX/TX-CL (92.2%) combination of both TZ and TX ESBL Etests (96.4%).

Conclusion: Etest PM/PML appears as the best single phenotypic confirmatory Etest performance for ESBL detection in this collection of Enterobacteriaceae containing a large proportion of AmpC producing isolates. Combination of TZ-TZ-CL and TX-TX-CL Etest strips is well suited for the detection of ESBLs in *E. coli* and in *Klebsiella* spp.

P888 Rapid detection of extended-spectrum β-lactamase production

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Objectives: The aim of this study was to create a method for the rapid detection of extended spectrum β-lactamases (ESBLs) from Gram-negative culture isolates using AK rapid technology. Adenylate kinase (AK) is novel and sensitive microbial cell marker which can be used to rapidly detect the growth of microorganisms without the need for conventional culture. The rapid AK method results would be compared to a phenotypical ESBL-confirmatory assay which takes 18–24 hours.

Methods: A panel of culture isolates comprising ESBL positive and negative strains were screened by inoculating two MIC-Strip ESBL test strips obtained from Merlin Diagnostics (Bornheim, Germany) according to the manufacturers instructions. The first strip was incubated overnight at 35–37°C and the result read visually. The phenotypical confirmation test is a $\geqslant$3 two-fold decrease in MIC of the suspected organism to cefpodoxime in the presence of a fixed concentration of clavulanic acid, versus its MIC when tested alone. The second strip was incubated at 37°C for 5 hours after which 0.05 mL was removed from each well, diluted 1:4 in Mueller-Hinton II broth and 0.1 mL of this diluted sample was then added to a microtitre plate.

The samples were then processed on a microplate luminometer with automated injection of the following reagents:

0.05 mL mixed bacterial lysis agent/purified ADP – for a 5 minute incubation time the adenylate kinase in the sample converts ADP to ATP.

0.05 mL Luciferase/Luciferin Reagent – followed by a 1 second read.

The results of each sample were then assessed according to an in-house devised algorithm and classified as 'Growth' or 'No Growth' and interpreted in the same way as the MIC-Strip ESBL test.

Results: There was 100% agreement between the rapid AK method and the MIC-Strip ESBL for all the organisms tested. All the ESBL positive and negative strains were correctly identified by both methods.

Conclusion: This new rapid method correctly predicted a positive/negative result for phenotypical ESBL confirmation within 5 hours.

P889 Comparison of VITEK 2 generated extended spectrum β-lactamase phenotypes to double-disc test results in Enterobacteriaceae

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Objectives: The aim of the study was to compare results of extended spectrum β-lactamase (ESBL) detection suggested by ESBL phenotypes generated by VITEK 2 (bioMerieux) advanced expert system (AES) to the results of double disk tests (DDT).

Methods: We retrospectively studied AES generated phenotypes suggesting ESBL presence in Enterobacteriaceae and results of DDT routinly performed to confirm the presence ESBL enzyme. Modified DDT was done with cefepime, cefotaxime and amoxicillin+clavulanic acid disks. AES phenotypes which included ESBL alone and/or ESBL together with other β-lactams phenotypes were taken into analysis. Analysis was performed by our laboratory information system – Promic (Antrez).

Results: From May to November 2007 we performed 928 Enterobacteriaceae susceptibility tests using VITEK 2 cards GN021 or GN041. In 140 isolates – among others 46 *Enterobacter cloacae*, 29 *Escherichia coli*, 16 *Klebsiella pneumoniae*, 13 *Citrobacter freundii* – AES suggested detection of ESBL phenotype although DDT confirmed its presence in only 66 isolates. The highest agreement between the AES and DDT was in *E. coli* (100%) and *Klebsiella* spp. (99%). The least correlation was noted in *E. cloacae* (46 phenotypes/6 confirmed), *Morganella morganii* (10/1) and *Serratia marcescens* (10/2). In 64 isolates when AES suggested ESBL phenotype as a sole β-lactams phenotype DDT confirmed ESBL production in 48 of them. In this case agreement between the AES and DDT with regard to species was the same.

Conclusion: VITEK 2 AES proposed phenotypes which suggest ESBL presence do not need confirmation in *E. coli* and *Klebsiella* spp. The same phenotypes in chromosonal cephalosporinase producers like *Enterobacter*, *Citrobacter*, *Serratia* and *Morganella* always need confirmation by another method for example modified DDT.

P890 Performance of VITEK 2 ESBL confirmation test in a set of well characterised clinical isolates of *E. coli*, K. pneumonia, *K. oxytoca* and *Enterobacter* species. An alternative for double disc testing

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Objectives: Severe infections caused by ESBL-producing microorganisms are becoming more common all over the world. Rapid and accurate ESBL detection has to be pursued by routine clinical laboratories in order to offer assistance in guided antibiotic therapy. Phenotypic ESBL confirmation tests must be performed on isolates that, based on initial antimicrobial testing, are suspected to produce ESBL. We evaluated the performance of the VITEK 2 ESBL confirmatory test on two sets of clinical isolates.

Methods: Strains; First set: 31 *E. coli* (ECO), 3 *Klebsiella pneumoniae* (KPN), 1 *Klebsiella oxytoca* (KOX), 19 *Enterobacter* spp., ESBL positive (+) and 7 *Enterobacter* spp. ESBL negative (–) confirmed by molecular methods. Second set: 50 *Enterobacter* spp. ESBL(–) or (+), phenotypic confirmation with Double Disk Synergy Test (DDST), Double Disk Combination Test (DDCT) and ESBL E-test. Confirmatory tests: VITEK 2 ESBL card (in conjunction with VITEK 2 GNS N048), DDST, DDCT and E-test cefepime.

Table 1.

Strains	Sequencing		DDST/DDCT/E-test		VITEK 2 ESBL test	
	ESBL+	ESBL–	ESBL+	ESBL–	ESBL+	ESBL–
First set						
E. coli (31)	31		28	3	28	3
K. pneumoniae (3)	3		3		3	
K. oxytoca (1)	1		1			1
Enterobacter (26)	19		18	1	17	2
		7		7	1	6
Second set						
Enterobacter (50)	ND			27		27
	ND		10*		10*	
	ND			13*	13*	

* MICs cefotaxime and ceftazidime >1 mg/L.

Results: see Table 1. Three out of 31 ECO were misidentified (2/3 SHV-12, 1/3 CTXM-2) with VITEK 2 ESBL test as well as with DDST,

DDCT and E-test. All *Enterobacter* isolates ESBL (+) strains, that were confirmed by PCR and sequencing and/or by phenotypic confirmation, were correctly identified with the VITEK 2. One sequenced *Enterobacter* spp. ESBL (–) was false positive with the VITEK 2. In 13 out of 40 ESBL (–) strains (phenotypic confirmation), VITEK 2 was positive.
Conclusion: For the ECO, KPN the VITEK 2 ESBL test performed as well as the DDST, DDCT and E-test. For *Enterobacter* isolates performance of the VITEK 2 ESBL test needs further evaluation.

P891 Effect of Oxgall on the imipenem-disc Hodge test in screening metallo-β-lactamase-producing Gram-negative bacilli

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Objectives: Increasing prevalence of carbapenemase-producing Gram-negative bacilli (GNB) has been a concern, but only a few phenotypic screening tests have been reported. We have been using the imipenem (IPM)-disk Hodge (cloverleaf) test to screen metallo-β-lactamase (MBL)-producing GNB. As the original Hodge test using Mueller-Hinton agar (MHA) was low in reproducibility, we now use MacConkey agar with much improved results. We investigated responsible ingredient that improve the results with MacConkey agar.
Methods: Materials used were MHA, MacConkey agar, 10-ug imipenem disk, Oxgall, starch and lactose. Bacterial strains used were those with known β-lactamases and clinical isolates. The surfaces of test media were inoculated using *E. coli* ATCC 25922 as was the CLSI disk diffusion test. After drying the surface, test organism was heavily streaked from the centre to the periphery of the plate and an IPM disk was placed at the centre. After overnight incubation, decrease of inhibition zone along the streaked line was measured and decrease of ⩾3 mm was arbitrarily interpreted as positive.
Results: Preliminary comparison using VIM-2-producing *Pseudomonas aeruginosa* (PAE, n = 4) and *Acinetobacter* spp. (ACI, n = 3) showed that 3 of 7 isolates were Hodge test positive by using MHA, but all were positive by MacConkey agar. Addition of 1% lactose or starch to MHA did not change the results. With 3 IMP-1-producing and 4 VIM-2-producing isolates, addition of 0.2% Oxgall did not improve the results, but addition of 2% changed the mean inhibition zone from 4.2 mm to 8.7 mm. OXA type carbapenemase-producing ACI gave positive results, although the reduction of inhibition zone was less than those with MBL-producing isolates. MHA-containing 2% Oxgall showed positive results with 26 of 28 OXA-type carbapenemase-producing ACI. On MacConkey agar, ESBL- or plasmid-mediated AmpC-producing isolates did not show positive Hodge test. blaTEM-107-cloned *E. coli* showed that the β-lactamase activity was much higher when grown in the Oxgall 2 mg/ml-containing ZYP-5052 medium, suggesting bile may enhance release of β-lactamase into the surrounding medium.
Conclusion: Bile is the ingredient, which improve performance of imipenem-disk Hodge test in the detection of MBL-producing isolates.

P892 Comparison of performance of MacConkey agar and Mueller-Hinton agar in imipenem-Hodge test

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Objectives: Imipenem-Hodge test for screening of carbapenemases is usually performed with Mueller-Hinton agar (MHA). However, some metallo-β-lactamases (MBL) producing strains show negative results. The aim of this study was to compare the performance of MacConkey agar (MCA) and MHA in imipenem-Hodge test.
Methods: The strains comprised 21 MBL-producers (14 IMP-producing *Pseudomonas aeruginosa*, 3 VIM-producing *P. aeruginosa*, 1 IMP-producing *Acinetobacter baumannii*, 1 VIM-producing *A. baumannii*, 1 SIM-producing *A. baumannii* and 1 VIM-producing *Alcaligenes faecalis*) that were not clearly positive from imipenem-Hodge test with MHA. Twenty nine isolates of non-carbapenemase-producing Enterobacteriaceae (AmpC β-lactamase producers, and/or ESBL producers) were also tested. The surfaces of MHA and MCA plates were inoculated with a lawn of indicator strain, *Escherichia coli* ATCC 25922, according to CLSI disk diffusion method. After drying the agar surface, a test strain was heavily streaked from the centre of the plate to the periphery and a 10 μg imipenem disk (Becton Dickinson) was placed at the centre. After overnight incubation at 35°C, presence of definite growth of indicator organism in the inhibition zone along the test strain was interpreted as positive. The two results from MHA and MCA plate were compared.
Results: With MHA, 9 of 21 MBL-producers showed negative results and 7 were weak positive, while definite positive results were obtained from only 5 strains. However, with MCA, 18 of 21 MBL-producers showed positive results and 3 were weak positive. All of the other non-carbapenemase producers were negative with MHA. However, with MCA, one AmpC-producing *E. coli* showed clearly positive indentation.
Conclusions: Imipenem-Hodge test with MCA were more sensitive and easier to read the results. The use of MCA should be helpful to clinical laboratories for the detection of MBL-producing bacteria.

P893 Prevalence and detection of metallo-β-lactamase-producing *Pseudomonas aeruginosa* strains from clinical isolates in Iran.

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Introduction: *Pseudomonas aeruginosa* is an opportunistic human pathogen and a cause of nosocomial infection, especially in immunocompromised patients. Carbapenems mainly imipenem and meropenem are potent agents for the treatment of infections caused by multidrug-resistant *P. aeruginosa*. Resistance to carbapenems in *Pseudomonas aeruginosa* is often due to loss of the OprD porin. The up-regulation of Mex AB-OprM efflux pump system compromises the activity of antibiotics including meropenem. The production of MBL is another mechanism for resistance to β-lactams including carbapenems.
Materials and Methods: The 126 isolates used in this study were selected from variety clinical specimens collected during 2006 at two university hospitals in Tehran, Iran. The isolates were identified by standard laboratory methods as *Pseudomonas aeruginosa*.The MICs for antimicrobial agents were determined according to the guidelines of the National Committee for Clinical Laboratory Standards. *P. aeruginosa* strains that showed resistance or reduced susceptibility to imipenem and/or ceftazidime were selected for further evaluation of MBL production by Etest MBL strips.A reduction of imipenem or ceftazidime MICs by 3 two fold dilution in the presence of EDTA was considered as positive.PCR analysis for detection of VIM-type MBL and IMP-type MBL was carried out with specific primers. Positive controls (MBL-producing strains)were used for PCR and Etest MBL strips.
Results: Of the 126 nonduplicate isolates tested, seventy strains that showed resistance or reduced susceptibility to imipenem and/or cetfazidime, were selected. Seventy isolates that showed resistance or reduced susceptibility to imipenem and/or ceftazidime were screened for presence of MBLs by Etest MBL strips. Etest MBL strips showed a positive results for eight *P. aeruginosa* strains. Finally, the 8 strains that were positive by Etest MBL strips gave a positive result with PCR.These eight strains carried a bla VIM-type gene, and bla IMP-type gene not detected.
Conclusion: The results from this research demonstrated that approximately, MBL-positive strains had multidrug-resistant(MDR). These data demonstrate that VIM-type gene are the most prevalent MBLs among clinical specimens of *P. aeruginosa* in Iran. Reports of acquired MBLs are increasing world wide among both non fermenters and enterobacteria. Therefore, suitable therapeutic methods should be used to prevent the spread of these resistances.

P894 **Evaluation of the Cica-Beta Test for routine detection of AmpC cephalosporinase hyperproduction and extended-spectrum β-lactamase production**

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Objectives: Distinction between ESBL and AmpC cephalosporinase hyperproduction (AHP) among resistant strains of Enterobacteriaceae is not easy in routine with automated identification systems, because of the low performances of the expertise in recognising such phenotypes. Cica-Beta Test (CBT) a new chromogenic method is available for rapid detection of AHP, ESBL, and metallo-β-lactamases (MBL). In the present study, we evaluated the performances of CBT in the detection of AHP and ESBL.

Material and Methods: All isolates were tested on VITEK 2. ESBL detection was performed using chromID ESBL Agar and CBT-CVA, in comparison to combined double disks, according to CLSI guidelines. AHP was assessed by CBT-C compared to detection of synergy between 3rd-generation cephalosporins (3GC) and either boronic acid or cloxacillin. We also tested CBT-I as a screening method able to detect either AmpC, ESBL, penicillinases or MBL. CBT-MBL was not tested because of the low incidence of MBL-producing bacteria in our institution. Inclusion criteria for Enterobacteriaceae were defined by (a) a positive ESBL-screening or expertise on VITEK 2; (b) a resistance up to 2nd-generation cephalosporins in non-natural AmpC-producers or a resistance up to 3GC in constitutive AmpC-producers.

Result: Among 40 consecutive stains, 23 were ESBL+, 26 were AHP+. Sensitivity of CBT-CVA and CBT-C was 83% and 54% respectively, and specificity was 65% and 29%. The CBT-I detected a resistance mechanism in 72%. A discordance between a negative CBT-I and a positive CBT-CVA or CBT-C was encountered in 12 cases. ChromID ESBL was positive for all ESBLs, but the specificity was very low (41%).

Conclusions: The goal aimed by CBT as a screening test for rapid detection is not reached because of the importance to pick colonies at boundary of inhibition zone of a 3GC; however, these step requires 18–24 h of reincubation after primoculture. The CBT is difficult to read with few contrast between a positive result and a negative one. The CBT lacks robustness and isolates must be picked in 5 minutes after removing from the incubator, when the β-lactamase production is maximal. Finally, the test is very expensive, and shows poor performances. We observed discordances between the screening test and the specific CBTs. For those reasons, CBT is not useful in routine. In comparison, a chromogenic medium such as ChromID ESBL Agar remains a cost-effective screening test for ESBL with a maximal negative predictive value.

Carbapenem resistance – Part 1

P895 **First detection of transferable metallo-β-lactamases in the Netherlands**

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Objectives: Metallo-β-lactamases (MBLs) are of special importance due to their role in microbial resistance to β-lactam antimicrobial agents, especially carbapenems. The prevalence of *Pseudomonas aeruginosa* strains that produce MBLs is increasing worldwide. Currently, nothing is known on the prevalence of these strains in The Netherlands. We determined the prevalence and the type(s) of MBLs produced by strains of *Pseudomonas* species isolated at the VU University Medical Center, in Amsterdam, The Netherlands.

Methods: During the first 7 months of 2007, the susceptibility profiles of 650 isolates from 329 patients were analysed. Isolates with decreased susceptibility to imipenem- and/or meropenem were tested for the production of MBL with MBL E-test. Site of infection/colonisation, and location of the patient in the hospital at the moment of strain isolation were recorded. DNA fingerprinting of the strains was performed with amplified fragment length polymorphism analysis (AFLP) to determine clonal relations. Transfer of resistance was investigated with conjugation experiments. The presence of VIM MBL genes was determined by PCR with generic primers. Further molecular characterisation of resistance genes confirmed the resistance pattern.

Results: Of 329 patients, 12 (3.6%) carried a MBL-positive *Pseudomonas* strain. Six strains were associated with clinical infection. The urinary tract was the most common site of infection. Epidemiological and molecular analysis showed no indications for a clonal outbreak in our hospital. Results of the conjugation experiment showed that these resistance genes were transferable. VIM PCR confirmed the production of VIM MBL in *Pseudomonas* isolates of two patients.

Conclusion: To the best of our knowledge, this is the first report that describes infections caused by MBL-producing *Pseudomonas* spp. strains in The Netherlands. Although the prevalence of metallo-β-lactamases is yet low, these results signal the need for development of accurate diagnostic tests by clinical laboratories to detect the presence of these resistance genes, so that specific isolation precautions can be taken to control their dissemination.

P896 ***Pseudomonas aeruginosa*** **producing VIM-2 metallo-β-lactamase in Cantabria, Spain.**

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Background: Carbapenem resistance rates of *P. aeruginosa* (Pae) in Spain and in other countries are similar, but strains producing metallo-β-lactamases (MBL) are quite uncommon in Spain. We report Pae producing VIM-2 in Cantabria, Spain.

Methods: All Pae isolates intermediate/resistant (I/R) to imipenem (IMP) and/or meropenem (MPM) (1 per patient) in Jan2004-Dec2006 were tested for MBL production. Susceptibility studies were done by Microscan, ETest strips and microdilution (following CLSI instructions; $\geqslant$8-fold decrease in the MIC of IMP or MPM combined with 0.4mM EDTA plus 0.04mM 1,10-phenantroline, compared with the MIC of the carbapenem alone indicated MBL production). MBL encoding genes were detected by a multiplex PCR (Mendes R., et al, 2007); PCR and sequencing were used for identification of blaVIM-2 and characterisation of type 1 integrons. Clonal relation of isolates was tested by Rep-PCR. Hydrolysis of IMP was detected by spectrophotometry.

Results: The blaVIM-2 gene was detected by PCR in 10 isolates for which the MICs of IMP and MPM ranged 32–128 and 16–32 μg/ml respectively. In all cases the strains were I/R to ceftazidime, cefepime, gentamicin, tobramicin and ciprofloxacin. 9/12 strains were amikacin-R. All isolates, except 1, were aztreonam susceptible. All isolates had a single Rep-PCR pattern. Hidrolysis of IMP in MBL(+) isolates ranged 0.1622 to 0.2159 nmol/min/μg protein and was abolished in the presence of 5 mM EDTA. An integron with 5′CS-aac(6)-blaVIM-2-putative transposase-enconding gene-3′CS was observed.

Conclusions: We have documented in Northern Spain clonally-related multiresistant *P. aeruginosa* strains producing type-1 integrons encoding blaVIM-2 and aminoglycoside-modifying enzymes.

P897 **First report of a** ***Morganella morganii*** **clinical isolate producing VIM-2 carbapenemase**

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IMP and VIM metallo-β-lactamases (MBL) are the most frequent carbapenemases, hydrolysing all β-lactams except aztreonam. They are often encoded by gene cassettes inserted into class 1 integrons. They are mostly disseminated among Gram-negative non-fermenters and have been rarely found in Enterobacteria. In 2004, a *Morganella morganii* isolate was collected at the University Hospitals of Coimbra, Portugal, from a patient's urine with an unusual susceptibility profile for this species. The aim of this work was to investigate the mechanism responsible for the reduced susceptibility to imipenem observed in this

isolate. Susceptibility tests were confirmed by the disc diffusion tests. MICs were determined by Etest. A synergy test with imipenem and EDTA was performed for detection of a MBL phenotype. Conjugation experiments were done by the filter mating method. Plasmid isolation was performed by using Plasmid Midi kit (Qiagen). A multiplex PCR was used for detection of VIM and IMP genes. Class 1 integrons were detected with specific primers 5′CS and 3′CS.
The isolate was resistant to amoxicillin, cefoxitin, cefuroxime, norfloxacin, ciprofloxacin and susceptible to aztreonam and amikacin. It showed decreased susceptibility to cefotaxime, meropenem, imipenem (MIC= 3 mg/L) and ceftazidime (MIC= 6 mg/L). The double-disk synergy test with imipenem- and EDTA-containing disks suggested the presence of a MBL. A multiplex PCR with IMP and VIM specific primers with positive controls for VIM-2 and IMP-5 (1,2), and further sequence analysis of the unique amplicon ca 800 bp, confirmed the presence of a VIM-2. Combinations of 5′CS, 3′CS and VIM primers were used to study the surrounding genetic context. A class 1 integron was amplified, integrating a unique cassette of aadB allele, unexpectedly not the blaVIM. The amplicon obtained with 5′CS and VIM reverse primers carried the aacA7 cassette, followed by the VIM-2 cassette. It was not possible to amplify a segment with 3′CS primer, suggesting that this integron has an unusual structure. A plasmid >10 Kb was found and blaVIM was amplified, but all the conjugation experiments were unsuccessfully using *E. coli* K102 as recipient.
To our knowledge, this is the first identification of VIM-2 in *M. morganii* worldwide. Apparently, spread of this MBL through enterobacterial population is low and it remained in this hospital a sporadic case, but the low carbapenem MICs found can mask the real situation.

P898 First occurrence of carbapenem-resistant *Pseudomonas aeruginosa* isolates producing VIM-2 metallo-β-lactamase in a Tunisian university hospital

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Objective: To characterise the β-lactamase-associated mechanisms of resistance of carbapenem-resistant *Pseudomonas aeruginosa* isolates recovered at the University Hospital Sahloul in Tunisia during a two-years period.
Methods: Seventy-five non-repetitive carbapenem-resistant *P. aeruginosa* isolates were recovered from November 2003 to May 2006. Metallo-β-lactamase (MBL) genes were searched by phenotypic and genotypic analyses, in particular genes for IMP-1, VIM-1 and VIM-2. Genotyping was done by ERIC-PCR. Amplification of class 1 integrons was performed by PCR with specific primers.
Results: All *P. aeruginosa* isolates were resistant to most β-lactams tested including imipenem. Five *P. aeruginosa* isolates possessed the blaVIM-2 gene. They had been isolated from blood culture and urine of patients hospitalised in the intensive care units or urology ward. Genotyping revealed that the VIM-2-positive *P. aeruginosa* isolates belonged to a single clone. The blaVIM-2 gene was likely located on the chromosome of those strains, and found to be inserted in a class 1 integron. The other carbapenem-resistant *P. aeruginosa* isolates (corresponding to three distinct clones) did not produce any carbapenemase.
Conclusion: This is the first report of dissemination of VIM-2 in *P. aeruginosa* in Africa. The occurrence of MBL determinants among *P. aeruginosa* isolates in Tunisia is worrying and further underlines the wide spread of VIM-2 worldwide.

P899 Genetic characterisation of the new blaVIM-18 and comparative antimicrobial susceptibility profile of VIM-18 with highest homologous VIM-variant, VIM-2: report from the SENTRY Antimicrobial Surveillance Program

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Objective: To characterise the genetic context of a new blaVIM-variant from India; and to compare the antimicrobial susceptibility (S) profiles of *E. coli* carrying recombinant plasmids harbouring blaVIM-18 and blaVIM-2. We recently found that VIM-producing *P. aeruginosa* were highly prevalent in Indian medical centres (55% of the carbapenem [CARB]-resistant [R] isolates) and VIM-2 was the most common VIM-variant (38.6%).
Methods: As part of the SENTRY Program, CARB-R isolates were screened for metallo-β-lactamases (MBL). Positive isolates were then amplified and sequenced with MBL internal primers and primers targeting conserved structures of class 1 integrons. blaVIM-2 and the new blaVIM were amplified, cloned into PCRScript plasmid and transformed in *E. coli* XL1 Blue. Colonies were selected with chloramphenicol (30 mg/L) and ceftazidime (4 mg/L). The presence and orientation of blaVIM was confirmed by PCR and sequencing. S testing was performed by E-test (ABBIODISK, Solna, Sweden). The region flanking the MBL integron was amplified using a degenerate primer approach. Plasmid contents analysis was also carried out.
Results: *P. aeruginosa* isolate 243–31C was recovered from a sputum specimen in Kolkatta, India (2006). PCR and sequencing showed the presence of new blaVIM variant, named blaVIM-18 (Lahey Clinic). The deduced VIM-18 protein comprised 262 amino acids showing 74.3 to 99.2% identity to other VIM enzymes. VIM-18 was identical to VIM-2, except for the deletion of four amino acids. The MBL active sites were all present. The *E. coli* carrying blaVIM-18 showed lower MIC values for ampicillin, cefoxitin, cephalothin and ertapenem (3-, $\leqslant$6-, $\leqslant$4- and 4-fold, respectively) when compared to those obtained from the *E. coli* carrying blaVIM-2, while similar MIC values were noted for imipenem, meropenem, piperacillin, cefotaxime, ceftazidime and cefepime. blaVIM-18 was the only R gene cassette in a class 1 integron that was flanked upstream by a resolvase gene (tnpR) from Tn5051-like. Further analysis showed that this isolate carried no plasmid.
Conclusions: VIM-18 displayed reduced activity to some β-lactams, while retaining similar activity against anti-pseudomonal agents when compared to VIM-2. Thus, the 12-bp deletion in VIM-18 seems to have little effect in the activity against target CARB and anti-pseudomonal cephems. VIM-18 was found in one *P. aeruginosa* isolate among numerous VIM-2-producing strains, and it appears to be an evolutionary derivative.

P900 High carbapenem resistance among *Pseudomonas aeruginosa* from India: a national epidemic of multiple metallo-β-lactamase clones (VIM-2, -5, -6, -11 and new VIM-18)

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Objectives: We assessed the prevalence of MBL-encoding genes among carbapenem-resistant *P. aeruginosa* isolates recovered in India during 2006. In addition, class 1 integrons harbouring MBL genes were amplified and compared and MBL-positive isolates were molecular typed to evaluate clonal dissemination.
Methods: A total of 282 *P. aeruginosa* were consecutively collected in 10 Indian medical centres and tested by CLSI broth microdilution methods. Isolates resistant to imipenem or meropenem (MIC, >8 mg/L) were screened for MBL-encoding genes by Real-time PCR. Positive samples were tested with primers targeting the class 1 integron conserved structures anchoring in the MBL genes. Amplicons generated were sequenced on both strands and MBL isolates ribotyped for possible clonality.

Results: Among the 282 isolates, 96 (34%) showed carbapenem resistance and MBL genes were detected in 53 strains (19% of total; 55% of carbapenem-resistant). MBL-producing *P. aeruginosa* were detected in 9 of 10 hospitals. Five blaVIM genes were found, including a new variant named blaVIM-18. This new MBL gene showed a 12 bp deletion (position 428) when compared to blaVIM-2 and was carried as a single gene cassette in a class 1 integron. VIM-2 producing isolates were most common (38 strains) and were detected in 8 medical centres. VIM-6 was identified in 12 isolates from 4 sites. VIM-5, VIM-11 and VIM-18 were found only once. Two medical centres had 3 distinct MBL types and other 2 had 2 MBL types. Wide genetic diversity was noted among MBL-carrying *P. aeruginosa* with 21 and 10 ribotypes seen among VIM-2- and -6-producers, respectively. Seven clones were found in ⩾1 participant hospital and 6 clones were noted within institutions. blaVIM-6-carrying integrons of 3.5 and 5 Kb (5 and 7 isolates, respectively) were detected and 2 sites had both blaVIM-6 integron types.

Table.

Site	CARB-R[a]/ MBL (%)	MBL types (No. of isolates)	MBL clonality		
			Intra	Inter	No. of clones
Kolkatta	30/22	VIM-2 (3), -5 (1), -18 (1)	0	2	2
Indore	42/42	VIM-2 (3), -6 (5)	1	2	3
New Delhi	34/18	VIM-2 (8)	1	3	4
Trivandrum	11/11	VIM-6 (2)	1	0	1
Hyderabad	51/17	VIM-2 (3), -6 (2)	1	3	4
Chennai[b]	46/0	None	–	–	–
Kochi	30/20	VIM-2 (5)	0	2	2
Manipal	30/20	VIM-2 (10)	2	3	5
Mumbai	30/22	VIM-2 (3), -6 (2), -11 (1)	0	3	3
Rajkot	25/25	VIM-2 (4)	0	1	1

a. CARB-R: carbapenem-resistant; b. Clones of non-MBL CARB-R *P. aeruginosa* were documented and VIM-2 by Toleman et al. (2007) from a 2003 isolate.

Conclusions: MBL-producing isolates have recently been reported in India, but limited data exists on the prevalence and characterisation of MBL-encoding genes. In this study we show that MBL-producing *P. aeruginosa* are epidemic in India with a great diversity of MBL-types (5 VIM types, including new VIM-18). In addition, different MBL-carrying integrons were observed, suggesting widespread dissemination of MBL-carrying mobile elements. Carbapenem resistance rates among *P. aeruginosa* were elevated and were principally caused by MBL production (57%).

P901 blaVIM-2-carrying integron among Enterobacteriaceae isolates in Mexico: report from the SENTRY Antimicrobial Surveillance Program

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Objectives: To characterise metallo-β-lactamase (MBL)-producing Enterobacteriaceae (ENT) strains isolated in a Mexican hospital and to evaluate the genetic context of the MBL-encoding genes. Carbapenems are generally very active against ENT but resistance has been emerging and has increased rapidly in some geographic areas due to the dissemination of MBL-carrying integrons.

Methods: ENT collected as part of the SENTRY Antimicrobial Surveillance Program were susceptibility (S) tested against >25 antimicrobials by CLSI broth microdilution methods. Isolates with imipenem and/or meropenem MIC at ⩾2 mg/L were screened for MBL production by the disk approximation test; positive isolates were further characterised by PCR and gene sequencing. Primers targeting the class 1 integron conserved structures anchoring MBL genes were used to reveal integron structure. Amplicons generated were sequenced on both strands and MBL-producing isolates were evaluated for possible clonality by PFGE.

Results: Three isolates, all from one hospital in Mexico, exhibited positive MBL-screening test results. Two *E. cloacae* (2005 and 2007) and one *K. oxytoca* (2006) yielded a PCR product with blaVIM primers. The MBL gene was embedded in a 2.8-Kb class 1 integron in all strains. Sequencing revealed that blaVIM-2 was located in the first position of the integron followed by 2 gene cassettes: an open reading frame (orf) and an aminoglycoside acetyl transferase gene (aacA7). The 400-bp orf showed low homology with other DNA sequences; however the deduced amino acid sequence showed active domains of a chorismate mutase enzyme from *Pseudomonas fluorescens*. The degree of carbapenem resistance varied significantly among these VIM-2-producing isolates. The *E. cloacae* isolates were epidemiologically unrelated and one strain was S to both imipenem and meropenem (ertapenem also S; 0.12 or 0.25 mg/L) based on current CLSI breakpoints of ⩽4 mg/L (see Table).

Organism	MIC (mg/L)[a]: IMI	MER	ERT	CPM	AMK	TET	CIP	TIG
K. oxytoca (13268A)	>8	>8	>8	4	⩽4	4	1	0.25
E. cloacae (3876A)	4[b]	1[b]	0.25[b]	2	⩽4	⩽2	⩽0.03	0.5
E. cloacae (1068D)	8	1[b]	0.12[b]	2	8	>8	0.5	0.5

a. IMI = imipenem, MER = meropenem, CPM = cefepime, AMK = amikacin, CIP = ciprofloxacin, TIG = tigecycline.
b. Indicates susceptible level carbapenem MIC results.

Conclusions: The finding of distinct ENT strains over a period of 3 years harbouring the same blaVIM-2-carrying integron indicates that this mobile genetic structure has become locally endemic. MBL-producing ENT may not be detected by clinical microbiology laboratories due to S-level carbapenem and other β-lactam MIC results, requiring new detection strategies or revised S breakpoints.

P902 Molecular typing indicates an important role of two international clonal complexes in the dissemination of VIM-producing *Pseudomonas aeruginosa* clinical isolates in Hungary

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Objectives: VIM metallo-β-lactamase-producing serotype O11 or O12 *Pseudomonas aeruginosa* isolates infecting or colonising 19 patients from seven hospitals were reported from Hungary between 2003 and 2005. In this work we identified VIM-producing *Pseudomonas* spp. strains from Hungarian towns and hospitals where these resistance determinants had not been detected before. Our aim was to characterise these isolates by molecular and phenotypic tools and to investigate their epidemiological relationship.

Methods: We screened the isolates by EDTA-inhibition based phenotypic tests. blaVIM genes and class 1 integrons were detected by PCR and were sequenced. PFGE was performed by the method described by Poh et al. with modifications and MLST was carried out according to the protocol published by Curran et al.

Results: The newly identified *P. aeruginosa* MBL producing isolates could be assigned to serotypes O1, O11 and O12. All isolates proved multidrug-resistant; however, it is notable that all *P. aeruginosa* isolates remained susceptible to aztreonam by the current CLSI breakpoints. VIM-producing *Pseudomonas* spp. clinical isolates from two novel locations and hospitals in Hungary were identified, with the detection of three new blaVIM carrying integron types and the presence of the blaVIM-2 allele in Hungary. By applying various typing techniques

including multilocus sequence typing we revealed an important role of two international clonal complexes in the dissemination of blaVIM positive *P. aeruginosa* in hospitals in Hungary. We characterised a *P. aeruginosa* nosocomial clone with a singleton sequence type (ST313), too, that could have acquired blaVIM-2 and blaVIM-4 gene cassettes from a yet unidentified local gene pool in Hungary.

Conclusions: This is the first report of the occurrence of *P. aeruginosa* isolates from Hungary belonging to an international clonal complex initially described as BG11. In this study we have demonstrated a high diversity of MBL carrying integrons in Hungary and also propose that a combination of different factors, such as clonal spread and local acquisition of MBL genes may both contribute to the appearance of VIM positive *Pseudomonas* spp. strains in our country. This work was supported by the DRESP2 FP6 project.

P903 Dissemination of VIM-1- and VIM-4-producing *Acinetobacter baumannii* clones in an intensive care unit

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Objectives: *Acinetobacter baumannii* complex are opportunistic pathogen with increasing relevance in hospital infections, especially among patients hospitalised in intensive care units (ICUs). The organism often exhibits multidrug resistance and recently outbreaks of carbapenem-resistant isolates have been reported worldwide. Acinetobacters develop resistance to carbapenems through various mechanisms including production of metallo-β-lactamases (MBLs). In Europe, carbapenem resistance has been only sporadically attributed to the production of MBLs. We describe the dissemination of two different VIM-type MBLs among clonal carbapenem-resistant *A. baumannii* isolates in a Greek ICU.

Methods: During a two year period (April 2005-March 2007) 24 MBL-producing carbapenem-resistant *A. baumannii* isolates were collected consecutively from clinical specimens of separate patients hospitalised in a medical-surgical Greek ICU. The isolates were tested by MICs, phenotypic MBL testing and PFGE. PCR and sequencing assays were used for identification of carbapenemase genes and mapping of integrons. The location of the MBL genes was investigated by PCR, curing and hybridisation experiments.

Results: The MBL-producers had multidrug-resistant phenotypes and clustered in two distinct clones. All 19 isolates of the predominant clone contained blaVIM-1 along with blaOXA-58 and the intrinsic blaOXA-66 carbapenemase gene. The remaining 5 isolates belonged to a distinct clone and contained blaVIM-4 along with the intrinsic blaOXA-69 carbapenemase gene. In all cases blaVIM alleles were integrated in class 1 integron variable structures. *A. baumannii* isolates belonging to the two VIM-producing clones were also detected in the environment of the ICU. PCR, curing and hybridisation experiments indicated that the blaVIM alleles were chromosomally-located.

Conclusions: This study illustrates the spread of blaVIM-1 and blaVIM-4 MBL genes among different clones of multidrug-resistant A. buamannii in a well defined clinical setting.

P904 Prevalence of the blaIMP-1 gene in epidemiologically unrelated *Pseudomonas aeruginosa* isolates in Korea

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Objectives: Resistance to carbapenems is reported increasingly in *Pseudomonas aeruginosa* worldwide. This study investigated the resistance mechanisms against carbapenems and the molecular epidemiology of carbapenem-resistant *Pseudomonas aeruginosa* isolates from a Korean hospital.

Methods: A total of 79 carbapenem-resistant *P. aeruginosa* isolates were obtained from a tertiary hospital, located in Daegu, Korea. Antimicrobial susceptibility was determined by agar dilution methods. Resistance to carbapenems was characterised by a carbapenemase activity test and PCR amplification. The oprD gene was screended by PCR and the expression of OprD protein in the outer membrane was determined by SDS-PAGE. PFGE was performed to determine the clonal relatedness of *P. aeruginosa* isolates.

Results: The resistance rates of metallo-β-lactamase (MBL) producers against β-lactams, aminoglycosides and fluoroquinolones were significantly higher than those of MBL non-producers. Of the 79 carbapenem-resistant *P. aeruginosa* isolates, two MBL genes were detected in 50 (63.3%) isolates: blaIMP-1 in 40 isolates and blaVIM-2 in 10 isolates. Both the blaIMP-1 and blaVIM-2 genes were inserted in the gene cassettes of class 1 integrons, which were located on the plasmids. The oprD gene was not amplified in the 43 isolates: 23 of the 50 MBL producers and 20 of the 29 MBL non-producers. The remaining 36 oprD-positive isolates showed the reduced expression of OprD in the outer membranes. PFGE suggests that carbapenem-resistant *P. aeruginosa* isolates originated from the diverse clones.

Conclusion: We report the first outbreak of IMP-1-producing *P. aeruginosa* in Korea. A high prevalence of plasmid-borne MBL genes in epidemiologically unrelated *P. aeruginosa* isolates suggests the possibility of the intra- and interspecies dissemination of the MBL genes.

P905 Nosocomial outbreak caused by multidrug-resistant *Pseudomonas aeruginosa* producing IMP-13 metallo-β-lactamase in France

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Objectives: Acquired metallo-β-lactamases (MBLs) are emerging resistance determinants in *Pseudomonas aeruginosa* and other Gram-negative pathogens. Six different MBL-types: IMP, VIM, SPM, SIM, GIM and AIM, have been identified. IMP13 has only been described in Italy so far. Here, we describe the molecular characterisation of IMP13-producing *P. aeruginosa* strains from Brest, located West of France.

Methods: Carbapenem-resistant *P. aeruginosa* strains recovered were analysed for their DNA content (genomic or plasmid), by isoelectric focusing (IEF), imipenem hydrolysis, conjugation assays and pulsed-field gel electrophoresis of SpeI digested genomic DNA. PCR were performed for MBL-genes (IMP, VIM, SPM, SIM and GIM), blaKPC, blaTEM and blaSHV genes, the chromosomal class C β-lactamase blaAMPC and blaOXA-50 genes.

Results: An outbreak of *P. aeruginosa* showing a multidrug-resistant (MDR) phenotype (being susceptible only to colistin, amikacin, and fosfomycin) was observed, from February to May 2006 in the hematology ward of the hospital of Brest, France. The outbreak involved 4 patients with a total of 7 isolates, mostly from blood samples specimens. Analysis of isolates involved in the outbreak revealed MBL activity, and PFGE revealed clonal relatedness. A blaIMP-13 gene cassette inserted into a class 1 integron which also contained an aacA4 aminoglycoside resistance cassette encoding an AAC(6)-Ib enzyme was identified in all strains. The blaIMP-13-containing integron and its genetic environment was different from the epidemic IMP-13-producing *P. aeruginosa* isolate from Italy. The blaIMP-13 gene was chromosome-encoded. Epidemiological investigation revealed no recent trip to Italy of any of the patients and the only common point between these patients was their hospitalisation room. However, no overlap in hospital stay, nor the presence in the environment, since all the environmental samples were negative for MBL-*P. aeruginosa*, could explain cross transmission.

Conclusion: Unlike VIM-producing *P. aeruginosa* for which large nosocomial outbreaks have been reported in France, Greece and Italy this is the first report of a nosocomial outbreak caused by *P. aeruginosa* producing an IMP-type enzyme in France and the second description of an outbreak related to IMP-13-producing *P. aeruginosa* isolates.

P906 Metallo-β-lactamases type IMP associated with loss expression of OprD porins in nosocomial isolates of carbapenem-resistant *Pseudomonas aeruginosa*

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Background: Carbapenem resistance (CBPR) in *Pseudomonas aeruginosa* (Psa) is associated with metallo-β-lactamases (MBLs) production or AmpC activity combined with loss of the porin OprD. In addition overexpression of efflux pumps could confer CBPR. In this study we investigated molecular mechanisms of CBPR in Psa causing bloodstream infections in Mexican hospitals.
Methods: We analysed eight Psa strains characterised in our laboratory. Antimicrobial susceptibility testing was performed by disk diffusion and dilution methods following CLSI standards. MBLs activity was detected by EDTA (0.5M)-disk synergy test with MEM (10 mg) or IPM (10 mg) and by E-test-MBL. The MBL genes were characterised by PCR using specific primers for IMP, GIM, SPM and VIM, and sequencing analysis. Production of OprD porin was performed by SDS-PAGE from outer membrane proteins. PFGE and endonuclease restriction analysis was used to characterise clonal dissemination.
Results: The eight Psa strains were isolated from three different hospitals in Mexico: Hospital Civil de Guadalajara (3), Hospital OHoran, (4) and Hospital General de Mexico (1). All strains were 100% resistant to AMK, CAZ, CIP, FEP, GEN, IMP, MEM and the MIC to IMP was >256 mg/mL in 6/8 strains. MBLs activity was confirmed in all strains, however by PCR and sequencing analysis only four strains were IMP type and negative to the other types. Three of these IMP-expressing strains failed to produce OprD porin. The PFGE profile demonstrated clonal variability.
Conclusions: Although isolates analysed are few, this is the first study that confirmed multiresistance, including CBPR in Psa strains causing serious nosocomial infections selected in different geographic areas in Mexico.

P907 Molecular epidemiology of *Pseudomonas aeruginosa* producing IMP-9 type metallo-β-lactamase isolated in Guangzhou, China

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Objectives: To investigate the resistance mechanisms and the genetic relationship of imipenem-resistant *Pseudomonas aeruginosa* isolates recovered in some hospitals in Guangzhou.
Methods: Imipenem-resistant *P. aeruginosa* clinical isolates were collected in various wards of four hospitals from Mar 2005 to Mar 2007. The isolates were analysed by PFGE for genotype, Etest MBL, PCR and sequencing for carbapenemase-encoding genes.
Results: During the study period, 134 clinical isolates of imipenem-resistant (MIC 8 to >128 mg/L) *P. aeruginosa* were isolated, and were classified by PFGE typing into 40 clusters, based on UPGMA (unweighted pair group method with arithmetic averages) clustering method. Each hospital had its own major epidemic strains, cluster A strains was most common in Hospital A(43/66, 65.15%), and strains belonging to cluster B, C, D were isolated from Hospital B (22.6%), Hospital C(33.3%) and Hospital D (31.6%), respectively. Although there was no epidemic circulating in all 4 hospitals, cluster Q appeared both in Hospital B and D and cluster A also appeared both Hospital B and Hospital C. There was one isolate belong to independent with the same genotype found in various samples from hospital A environment survey, though six patients of lower-airway colonisation with clone A *Pseudomonas aeruginosa* had been admitted to the same ICU of hospital A for many times. Analysis of antimicrobial resistance of the epidemic strains from four hospitals, revealed that 44 of 45 cluster A isolates of *Pseudomonas aeruginosa* produced IMP-9 metallo-β-lactamase, with pI of 7.8. The bla gene located in class 1 integron, and none of cluster B, cluster C and cluster D isolates produced IMP-9 metalloenzyme.
Conclusion: The various degree of outbreak of Imipenem-resistant *Pseudomonas aeruginosa* had occurred in four hospitals individually in two years survey. Wide dissemination of IMP-9 metalloenzyme contributes partially to the imipenem resistance of *P. aeruginosa* isolates of cluster A. It was important to manger the patients colonised with epidemic strain only to prevent clonal transmission.

P908 Biochemical characterisation of the subclass B2 metallo-β-lactamase SfhI

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Objectives: Metallo-β-lactamases are characterised by their ability to hydrolyse almost all β-lactam antibiotics and are not inhibited by commonly used inhibitors (e.g. clavulanate, tazobactam). The β-lactamase SfhI was detected in strain *Serratia fonticola* UTAD54, an environmental isolate obtained from untreated drinking waters in the Northeast of Portugal. The aim of the present study was to purify this class B carbapenemase and determine its biochemical properties.
Methods: The blaSfhI gene was cloned into expression vector pET-26b(+) after PCR amplification using specific primers and the accuracy of the DNA construction verified by sequencing. The recombinant plasmid was transformed into *Escherichia coli* BL21(DE3). Metallo-β-lactamase SfhI was purified from induced cultures of *E. coli* BL21(DE3)(pSfhI) grown in M9Y medium supplemented with kanamycin at 25°C. Purification of SfhI was achieved by means of two chromatographic steps, anion-exchange at pH 8.5 followed by size exclusion at pH 7.0. Molecular mass and metal content of SfhI were determined by mass spectrometry. Initial hydrolysis rates for a range of β-lactam antibiotics were measured in 50 mM Hepes buffer pH 7.0 at 25°C.
Results: High level production of recombinant SfhI was achieved. Approximately 80 mg purified protein per liter of culture were obtained. By SDS-PAGE, protein was estimated to be >95% pure. On SDS-PAGE gels the purified SfhI migrates as a single band of approximately 26 kDa. Molecular mass of SfhI was determined as 26136 Da by mass spectrometry, in agreement with values predicted from the deduced amino acid sequence. The purified protein was shown to bind one equivalent of zinc, consistent with the assignment, on the basis of amino acid sequence, of SfhI to the B2 metallo-β-lactamase subgroup. Preliminary kinetic data suggest that SfhI hydrolyses penicillins, cephalosporins and carbapenems, exhibiting high affinity for carbapenems and low affinity for penicillins and third generation cephalosporins. Hydrolysis of cefoxitin and moxalactam was also detected.
Conclusion: The overexpression of blaSfhI allowed us to obtain a large amount of SfhI enzyme, enabling effective protein purification. Although the SfhI sequence is the most divergent (c.50% identity) of the known B2 metallo-β-lactamases, kinetic studies showed a substrate range that generally resembles those of other subgroup members, but includes cefoxitin and moxalactam, known inhibitors of these related enzymes.

P909 Hydrolysis parameters for doripenem, meropenem and imipenem tested with β-lactamases of molecular classes A to D

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Objectives: Doripenem (DOR) is a carbapenem with activity against Gram-negative pathogens including activity against *P. aeruginosa*. One of the mechanisms of resistance observed for carbapenems such as imipenem (IPM) and meropenem (MEM) is hydrolysis by β-lactamases with carbapenemase activity. The purpose of these experiments was to determine the interactions of doripenem and comparators with β-lactamases of molecular classes A to D.
Methods: β-lactamases were purified by gel filtration and ion exchange chromatography. Protein concentrations were measured by BioRad Protein Assay. Purity of β-lactamases was assessed by PAGE gels. Initial hydrolysis rates were measured spectrophotometrically, and kinetic

parameters were determined using a Hanes plot. MIC values were determined using CLSI broth microdilution methodology.
Results: Most Class A β-lactamases, including the ESBLs TEM-26 and CTX-M-15, demonstrated very low levels of hydrolysis for DOR, IPM and MEM. However, a subset of Class A enzymes, the serine carbapenemases KPC-2 and SME-3, showed hydrolysis of all carbapenems tested. For KPC-2, DOR (kcat value 0.55 s-1) and MEM (kcat 3.6 s-1) were hydrolysed 9–60-fold slower than IPM (kcat 31 s-1). For SME-3, DOR (kcat 2.4 s-1) and MEM (kcat 3.2 s-1) hydrolysis was approximately 100-fold slower than IPM hydrolysis (kcat 320 s-1). The Class B metallo-enzymes such as VIM-2 also hydrolysed DOR more slowly than IPM (kcat 2.4 s-1 and 20 s-1). The Class C P99 and Class D OXA-10 had negligible hydrolysis of all carbapenems (kcat <0.02 s-1).
Conclusions: Doripenem was stable to hydrolysis by ESBLs and AmpC-type β-lactamases. Hydrolysis of all three carbapenems was observed with the serine carbapenemases and metallo-β-lactamases. For the carbapenemases, DOR hydrolysis was 5–100-fold slower than IPM hydrolysis.

P910 Porins and ertapenem resistance in *Klebsiella* spp. from the UK

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Objectives: Although still rare, carbapenem resistance is increasingly detected in Enterobacteriaceae, with ertapenem often the most affected analogue. We investigated mechanism(s) of ertapenem resistance in clinical isolates of *Klebsiella* spp. referred to the UK's national reference laboratory.
Methods: MICs were determined by agar dilution or Etest. Isolates were typed by PFGE of XbaI-digested genomic DNA. Nine isolates highly resistant to ertapenem (MICs ⩾8 μg/ml) were investigated further. β-lactamase genes were identified by PCR. Phe-Arg-beta-naphthylamide (PABN) was used to test the contribution of efflux pumps. Porins were profiled by SDS-PAGE. Transcription of ompK35 and ompK36 porin genes and the acrB efflux pump gene was analysed by real-time reverse transcriptase PCR. Porin genes and their promoter regions were sequenced. Complementation analysis used a plasmid encoding OmpK36.
Results: The PFGE profiles of 30 ertapenem-resistant clinical isolates from 26 laboratories were heterogeneous. Imipenem and meropenem MICs ranged from 0.5 to 16 and from 2 to16 μg/ml, respectively. All 9 strains studied in detail produced ESBLs: CTX-M group1 (n = 3), CTX-M group1 and 9 (n = 3) and SHV (n = 3) were identified. Ertapenem MICs were reduced by ⩽2-fold in the presence of PABN, contraindicating efflux as a contributor to resistance. Isolates lacked OmpK35 and OmpK36, but there were no significant variations in the levels of expression of ompK35, ompK36 and acrB genes as compared with carbapenem-susceptible isolates. Sequencing ompK36 revealed various point mutations or insertion sequences (IS) within the Open Reading Frames (ORFs), predicted to result in early termination of translation. Sequencing of ompK35 identified a wild-type sequence for 2 isolates and an ORF disrupted by point mutation or IS10 for 2 others whereas, for 5 isolates, IS1 was identified in the promoter region or at the end of the ompK35 ORF resulting in a 10 amino acid deletion. Carbapenem MICs for all strains were reduced markedly when complemented with a plasmid carrying an intact ompK36 gene.
Conclusions: Ertapenem-resistant *Klebsiella* spp. from the UK represent diverse strains. Their major mechanism of ertapenem resistance is ESBL production in combination with porin loss, most importantly OmpK36 in conjunction with an ESBL.

P911 Characterisation of carbapenem-nonsusceptible *Escherichia coli* isolates from a university hospital in Taiwan

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Objectives: To investigate characteristics of carbapenem-nonsusceptible *Escherichia coli* isolates collected between 1999 and 2005 at a Taiwanese university hospital.
Methods: A total of 9 nonduplicate carbapenem-nonsusceptible *E. coli* isolates were examined. Genetic relatedness was analysed by PFGE. β-lactamases were characterised by PCR and isoelectric focusing. Outer membrane proteins and transcripts were investigated by SDS-PAGE and Northern blotting. Cloning experiments were performed to investigate the role of membrane permeability in carbapenem nonsusceptibility.
Results: The 9 carbapenem-nonsusceptible isolates were found to produce the CMY-2 AmpC enzyme (N = 8), the CTX-M-14-type extended-spectrum β-lactamase (N = 1), the SHV-12 extended-spectrum β-lactamase (N = 1), and the IMP-8-type metallo-β-lactamase (N = 1) alone or in combination. All carbapenem-nonsusceptibe isolates revealed a decrease in the transcription and protein expression of ompC, and susceptibility to carbapenems was restored in one isolate by introducing the cloned ompC gene. PFGE revealed genetic diversity among the 9 isolates. All patients with the carbapenem-nonsusceptible isolates had been treated with carbapenems (6 patients) and/or extended-spectrum cephalosporins (5 patients) before isolation.
Conclusions: Our study suggests that the decreased susceptibility to carbapenems in *E. coli* in the hospital might arise by the stepwise accumulations of multiple drug-resistance determinants in different clones.

P912 Carbapenem (cfiA) resistance gene in *Bacteroides fragilis* group strains in Belgium

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Objectives: In a recent Belgian survey [1] 3% of the *B. fragilis* group strains were not susceptible to meropenem. In our university hospital a decrease in susceptibility to meropenem was observed from 97% in 2005 to 92% and 93% in 2006 and the first 8 months of 2007 respectively. In isolates belonging to other species of the *B. fragilis* group isolates susceptibility to meropenem was 97% in 2006 and 100% in the other years. The purpose of this study was to determine the prevalence of the cfiA carbapenem resistance gene in *B. fragilis* group isolates collected for a Belgian survey for resistance in anaerobes and to look for the presence of the cfiA gene in meropenem non-susceptible strains of this group isolated in our hospital.
Methods: From October 2003 to February 2005 238 *B. fragilis* group strains (135 *B. fragilis* and 103 other species) were collected in 9 Belgian hospitals. Also included in this study were 15 meropenem non-susceptible strains isolated in our hospital. Meropenem susceptibility was determined by Etest®. The following breakpoints were used for susceptible and resistant strains: ⩽4 and ⩾16 mg/L. The strains were screened for cfiA gene by PCR with specific primers.
Results: Of 135 *B. fragilis* collected for the survey three showed reduced susceptibility (MIC=8) and three were resistant (1 with MIC=16 and 2 with MIC=32). All these isolates and three of four isolates with a MIC of 4 were cfiA gene positive. All *B. fragilis* isolates with a MIC less than 4, one *Bacteroides fragilis* isolate with a MIC of 4 and the 103 isolates from other species were cfiA gene negative.
Fifteen meropenem non-susceptible strains (MIC>4; 12 *B. fragilis*, 1 B. thetaiotaomicron, 1 B. ovatus and 1 B. vulgatis) were detected in our hospital since 2005. Eleven *B. fragilis* isolates were cfiA gene positive. The cfiA gene was not detected in the remaining *B. fragilis* strain (MIC=8) and in the other species.
Conclusion: The prevalence of the cfiA resistance gene in Belgian survey isolates (2003–2005) was 6.7% (9/135) in *Bacteroides fragilis* isolates. All except one of meropenem non-susceptible *B. fragilis* strains

isolated in our hospital since 2005 were cfiA gene positive. The cfiA gene was not detected in other species of the *B. fragilis* group.

Reference(s)

[1] Wybo. I. et al. Third Belgian multicentre survey of antibiotic susceptibility of anaerobic bacteria. Journal of Antimicrobial Chemotherapy 2007; 59: 132–9.

Molecular bacteriology

P913 Characterisation of the virulence markers of Shiga toxin-producing *Escherichia coli* isolated from beef samples

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Shiga toxin-producing *Escherichia coli* (STEC) strains are an important cause of bloody diarrhoea, haemorrhagic colitis and haemolytic uremic syndrome. Transmission of STEC occurs through consumption of undercooked meat. STEC often belong to O157:H7 however, several other serogroups have been identified. The pathogenicity of STEC is mediated by Shiga toxin (Stx) and the intimin. Recent studies described additional factors: 67-kDa IrgA, Efa1, LPF or Saa.

Objectives: To characterise the virulence markers of STEC isolated from beef.

Methods: The STEC were isolated from beef samples (n = 342) by the use of DNA hybridisation with DIG probes and then tested using the multiplex PCR (rfbO157, stx1, stx2, fliCH7, eaeA, ehlyA, O26wzx, rfbO111, rfbO113 and 16SrRNA) and PCR assays (O45wzx2, O55wzx2, O121wzx1, stx2c, stx2d, stx2e, stx2f, katP, iha, toxB, efa1, saa, lpfAO157/OI-141, lpfAO157/OI-154, lpfAO113, eae alpha and gamma).

Results: Sixteen stx-positive *E. coli* strains were detected, eight were O157:H7. The stx1 marker was observed in all *E. coli* O157:H7 and in four non-O157:H7 isolates. The stx2 gene was detected in all *E. coli* O157:H7 and in seven of non-O157:H7. The identification of Stx variants resulted in presence of the stx2d gene in 13 STEC and among them 3 strains had the stx2c marker. Among eight of the O157-negative STEC, three belonged the O26 group. These isolates also carried the stx1 (one strain) or stx2 genes (two isolates). None belonged to the O45, O55, O111, O113 or O121 groups. All harboured the enterohaemolysin (ehlyA) gene whereas the intimin marker (eaeA) was observed in all *E. coli* O157:H7 (alpha) and O26 isolates (gamma). The factors such as katP, toxB and efa1 were detected in all O157:H7 as well as in one O26 isolate. Fifteen STEC were the iha-positive and five the saa-positive. None of the isolates possessing the saa marker was O157 or O26. The presence of the lpfAO157/OI-141 and lpfAO157/OI-154 genes was noted among 8 O157:H7 and in one O26 STEC. The lpfAO157/OI-141 gene was present in 4 other STEC including *E. coli* O26. None of *E. coli* O157:H7 had the lpfAO113 marker that was observed in the all remaining STEC. In one isolate belonging to the *E. coli* O26 group, for the first time a simultaneous presence of the lpfAO157/OI-141, lpfAO157/OI-154 and lpfAO113 was noted.

Conclusion: The obtained results can provide a better knowledge of the genetic diversity of STEC strains as well as should lead to great progress in molecular epidemiology.

P914 Virulence potential of extraintestinal pathogenic *Escherichia coli* strains isolated from skin, wound and soft tissue infections

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Objectives: The bacteria *Escherichia coli* (*E. coli*) is an important facultative anaerobe of the intestinal normal flora, but pathogenic strains can cause an impressive variety of different types of intestinal and extraintestinal diseases. Strains causing infections differ from commensal *E. coli* due to possession of virulence factors. The best characterised pathogenic *E. coli* strains are those from infections of gastrointestinal tract, urinary tract, meningitis and sepsis. Some reports of *E. coli* strains involved in skin, wound and soft tissue infections can be found, however to our knowledge no survey of the virulence potential of these strains has yet been performed. Therefore, in the presented work, a collection of 122 extraintestinal pathogenic *E. coli* strains isolated from skin, wound and soft tissue infections were studied.

Methods: Isolates were obtained from the Institute of Microbiology and Immunology, Medical Faculty in Ljubljana, Slovenia. The phylogenetic group of each strain was determined and the strains were screened by PCR with primers specific for the following virulence (related) genes cnf1(cytotoxic necrotising factor 1), hlyA (haemolysin), subA (subtilase cytotoxin), papGII (class II PapG adhesin), papGIII (class III PapG adhesin), sfaDE (S fimbriae), afa/draBC (Afa/Dr adhesins), iucD (aerobactin), kpsMT (synthesis of group II capsule), ompT (outer membrane protease) and usp (uropathogenic-specific protein). Dot-blot hybridisation experiments were performed to validate the PCR assays.

Results: Our results showed that 66% of the studied *E. coli* isolates from skin, wound and soft tissue infections belonged to group B2, 12% to the group A, 12% to the group D and 10% to the B1 group. The cnf1 gene nucleotide sequences were detected in 34% of strains, hlyA in 31%, papGII in 16%, papGIII in 23%, sfaDE in 37%, afa/draBC in 1%, iucD in 50%, kpsMT in 65%, ompT in 20% and usp in 48% of the tested isolates. No strain harboured subA sequence.

Conclusion: Our results showed that the virulence potential of *E. coli* strains from skin, wound and soft tissue infections is comparable to the virulence potential of pathogenic *E. coli* strains from other extraintestinal infections (Table 1).

Table 1. Comparison of results from different studies on extraintestinal pathogenic *E. coli* isolates

	Prevalence (%)										
	cnf1	*hlyA/D*	*papGII*	*papGIII*	*sfa/foc*	*afa/dra*	*iucD/iutA*	*kpsMT*	*ompT*	*usp*	Ref.*
75 urosepsis isolates (USA)	16	41	71	7	25	9	nt**	63	nt	nt	1
170 pyelonephritis isolates (USA)	nt	39	60	9	nt	17	63	86	82	nt	2
74 cystitis isolates (USA)	34	36	5	31	36	4	nt	62	nt	nt	3
377 urinary tract infection isolates (Japan)	50	51	nt	nt	46	8	39	nt	nt	85	4,5
88 vaginal isolates (Japan)	19	22	nt	nt	20	6	35	nt	nt	nt	6
100 cystistis isolates (Israel)	34	44	31	17	37	14	nt	69	53	nt	7
110 urinary tract infection isolates (Slovenia)	23	25	34	13	24	2	42	nt	nt	44	8
122 skin, wound and soft tissue infections (Slovenia)	34	31	16	23	37	1	50	65	20	48	9

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8. Our unpublished data.
9. This study.

**nt = not tested.

P915 Cloning and expression of HpaA protein of *Helicobacter pylori*

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Introduction: *Helicobacter pylori* is associated with the chronic gastritis, peptic ulcer, gastric adenocarcinoma, and gastric mucosa-associated lymphoid tissue (MALT) lymphoma. The antibiotic therapies do not protect from potential re-infection, and have a risk for development of drug resistance. Therefore, the prophylactic vaccine mediated protection against *H. pylori* is an attractive clinical interest. *H. pylori* adhesin A (HpaA) is a conserved surface lipoprotein and plays important roles in pathogenesis of infection. In this study an hpaA gene cloned in pET expression vector and the recombinant protein (rHpaA) was over expressed in *E. coli*.

Methods: Genomic DNA of the standard *H. pylori* strain 26695 was isolated as the template and hpaA gene was amplified by PCR. Prokaryote expression vector pET28a inserted with hpaA gene (pET28a-hpaA) was constructed. The recombinant plasmid was used to transform competent *E. coli* DH5alpha, and positive clones were selected. Then the recombinant plasmid was used to transform *E. coli* BL21DE3 for

expression of recombinant protein HpaA. The expression of recombinant protein induced by isopropythio-beta-D-galctoside (IPTG) at different concentration was examined by SDS-PAGE. Western blot assay were used to determine immunoreactivity of rHpaA by a rabbit polyclonal antibodies against whole cell of *H. pylori*.
Results: In comparison with the reported corresponding sequences, the nucleotide sequence homology of hpaA gene was 98.8%. HpaA fusion protein was able to react with the rabbit polyclonal antibody against whole cell of *H. pylori*.
Conclusion: A prokaryotic expression system with high efficiency of *H. pylori* hpaA gene was successfully established and HpaA fusion protein showed satisfactory immunoreactivity. These results indicate that production of specific recombinant protein is an alternative and potentially more expeditious strategy for development of *H. pylori* vaccine.

P916 Characterisation of a *Streptococcus agalactiae* serotype Ia lineage with enhanced invasiveness

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Objectives: We have recently identified a lineage of group B streptococci (GBS) expressing serotype Ia as having enhanced potential to cause early-onset invasive disease in neonates (EOD). Characterisation by multilocus sequence typing (MLST) revealed that this lineage was characterised by two sequence types: ST23 and ST24. Data in the literature suggests that ST23 is not particularly virulent so we decided to further characterise this PFGE lineage regarding antibiotic susceptibility and the presence of the genes encoding the alpha and alpha-like family of surface protein, with the aim of clarifying the apparent heterogeneity within the lineage.
Methods: Susceptibility to penicillin, chloramphenicol, cefotaxime, tetracycline, vancomycin, erythromycin and clindamycin was tested by disk diffusion according to the CLSI guidelines and interpretative criteria. Macrolide-resistant isolates were further classified as having the cMLSB, iMLSB, or M phenotype by a double-disk diffusion test. A multiplex PCR reaction was set up to detect the presence of the erm(B), erm(A) and mef genes. Surface protein gene profiling was also obtained by a multiplex PCR assay targeting the bca, alp2, alp3, alp4, eps and rib genes.
Results: All isolates were fully susceptible to penicillin, chloramphenicol, cefotaxime, and vancomycin and resistant to tetracycline. Resistance to erythromycin was found in 3 isolates occurring simultaneously with clindamycin in two isolates. Of the 3 erythromycin-resistant isolates, 2 had the cMLSB and 1 the M phenotype. The isolate presenting the M phenotype carried the mef gene, whereas the erm(A) gene [erm(TR) variant] was found in the 2 cMLSB isolates. All isolates carried a single macrolide-resistance determinant. All isolates tested presented only the bca or eps genes, encoding the alpha-C and epsilon surface proteins, respectively. The bca gene was found associated exclusively with isolates representing ST24 and the eps gene only with isolates presenting ST23.
Conclusions: In spite of the mostly homogeneous susceptibility profile of the GBS lineage with enhanced potential to cause EOD, the complement of surface proteins further confirms the distinction in two sub-populations already suggested by the MLST analysis. The unusually high proportion of isolates representing ST24 and carrying the bca gene in our collection and not frequently found elsewhere, may have been decisive for the identification of the enhanced invasiveness of this lineage.

P917 Characterisation of cloning and in vitro expression of enolase from *Streptococcus suis* 2

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Objectives: To clone enolase (eno) gene and investigate some characteristic of its product Enolase (Eno) in *Streptococcus suis* serotype 2 (*S. suis* 2, SS2).
Methods: Using the Qiaexpress expression plasmids, the enolase gene was inducibly over expressed in *E. coli* to produce Enolase with a hexahistidyl N-terminus to permit its purification. Western-blot experiment and Enzyme-linked immunosorbent assay were used to detect its antigenicity. Whole cells ELISA was developed to give confirmation about Enolase localisation. Moreover, an adherence assay with Hep 2 cells was performed to determine the implication of the Enolase in the *S. suis* 2 adhesion. Action on lymphocytes designed to explore the role of Enolase in immunoreaction related to streptococcal infection.
Results: A highly homologous eno gene was unveiled by the genome-wide mining. The target DNA fragment of about 1.3 kb was successfully amplified using the genomic template of 05ZYH33 and protein expression analysis showed that a 75 kD protein can be observed in 12% SDS-PAGE, indicating that the recombinant 6His-fused Eno protein can be produced in *E. coli* under the induction of IPTG. Western-blot experiment demonstrated clearly it shares strong specific antigenicity. ELISA result suggested that Eno can occur on the surface of 05ZYH33 strain. Adherence assay showed a significant reduction in the adhesion of *S. suis* 2 in the 6His-fused Eno pre-incubated cells compared to the non-incubated cells. Evaluation of antibody titers in ELISA indicated that serum samples from pigs with SS2 infection have higher levels of antibodies that react with the recombinant Enolase than with whole bacteria sonication lysis and MRP. Apotosis of lymphocytes showed that streptococcal Enolase is a novel cross-reactive antigen that may play an important role in the initiation of the autoimmune diseases related to streptococcal infection.
Conclusion: The results exhibited the properties of Eno protein and provided an efficient Eno-based method for monitoring SS2 infection. Together, our data supported that Eno can function as a novel antigen, and may play pivotal roles in the severe infection of *S. suis* 2.

P918 Usefulness of denaturing high-pressure liquid chromatography to assess in situ gut microbiota in neonates

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Objectives: Microbiota colonisation of the neonatal gastrointestinal tract develops within a few hours after birth and major changes occur during the first postnatal weeks. Current knowledge of the gut microbial ecology is based on faeces studies, mainly by classical culture-dependent techniques. Nowadays, a new technique of fingerprinting, the DHPLC can be used. So far, however, no data is available for kinetics of implantation, growth and development of endoluminal microbiota in infants. Thus we did a prospective analysis of the in situ gut microbiota during neonatal surgery by classical cultures techniques, TTGE and DHPLC.
Methods: Newborns under 28 days of life requiring digestive surgery were included after parental information. During surgery, intestinal swabs were sampled. Then, the intestinal microbiota was analysed by conventional culture-based techniques and by two culture independent methods based upon bacteria DNA extraction and separation of amplified 16S rRNA gene fragments using Denaturing High Pressure Liquid Chromatography (DHPLC) and Temporale Temperature-Gradient Gel Electrophoresis (TTGE).
Results: 11 newborns (gestational age 33±2 weeks) were included. Two were born via caesarean section. Among the 6 newborns operated before 48 hours of life, 4 had a negative culture, no TTGE bands, three of which had a positive DHPLC analysis. The most frequent bacteria were Gram-negative bacilli (*Enterobacter* and *E. coli*), Gram-positive cocci (negative coagulase *Staphylococcus*-NCoS). Three were found with *Pseudomonas* (*P. aeruginosa*) and one with *Clostridium* (*C. butyricum*).
The three techniques used have shown coherent results. None uncultivable bacteria have been found.
Conclusion: Our work shows that DHPLC, based on DNA static analysis, is a powerful technology. DHPLC is a reproductible method that allows rapid characterisation of the bacterial establishment in neonates. DNA amplicons are easy to elute for sequencing. Moreover, it offers

full automation. The neonatal supra-mesocolic tractus is colonised very early in life mostly with Enterobacteria and NCoS.
These results could be helpful to design specific antibioprophylaxis guidelines in neonates and to monitor microbiota changes induced by various neonatal diets and treatments.
This work has been supported by Atlangene and Transgenomic

P919 Molecular evaluation of Brazilian vaginal lactobacilli microbiota and the role of hydrogen peroxide-producing isolates

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Objectives: To study vaginal lactobacilli populations by culture-dependent [polymerase chain reaction and amplified ribosomal DNA restriction analysis (PCR-ARDRA)] and culture-independent [PCR and denaturing gradient gel electrophoresis (PCR-DGGE)] methods, and to evaluate the ability of isolates to produce H2O2.
Methods: This study, approved by local Ethics, comprised three groups of women recruited in the city of Ribeirão Preto, São Paulo State, Brazil: 64 healthy, 68 diagnosed with vulvovaginal candidiasis (VVC) and 64 with bacterial vaginosis (BV). Samples from lateral vaginal wall were obtained with swabs, transferred to tubes containing physiological saline and decimally diluted. Dilutions were surface plated on de Man Rogosa Sharpe (MRS) agar and incubated for 48 h at 37°C (aerobiosis and anaerobiosis). Isolates characterised as Gram-positive rods, catalase and oxidase negative were identified by PCR-ARDRA and evaluated semi-quantitatively for H_2O_2 production (Merckoquant Peroxide Test; Germany). Swabs obtained from patients were also evaluated by PCR-DGGE for detection of lactobacilli.
Results: Lactobacilli were isolated from 87.5% of controls, 88.2% of subjects with VVC and 32.8% of women with BV using PCR-ARDRA, compared to 98.4%, 94.1% and 57.8% assessed by PCR-DGGE, respectively. By PCR-ARDRA, *L. crispatus* was the most prevalent isolate in control group (37.0%), followed by *L. johnsonii* (16.4%). In VVC patients' group, *L. crispatus* was also the most common isolate (35.9%) followed by *L. jensenii* (21.8%), whereas in the group of BV, *L. gasseri* (36.4%) and *L. crispatus* (18.2%) were more frequently found. According to PCR-DGGE, *L. iners* was the most prevalent species in all groups. Only *L. crispatus* was detected accordingly by both methods in the three groups (Kappa coefficient = 0.531). From healthy and VVC groups, 1.4% and 2.6% respectively of the lactobacilli isolates did not produce H_2O_2, whereas in the BV group, this rate raised to 31.8%. In both healthy and VVC groups, *L. crispatus* and *L. johnsonii* produced, comparatively, the highest average amounts of H_2O_2.
Conclusions: The evaluation o vaginal microbiota was dependent on the methodology employed and the results also suggest the absence of H_2O_2-producing lactobacilli in the vaginal microbiota may be a contributing factor for the development of BV, whereas the presence of these isolates may not be protective against VVC.
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P920 The occurrence of intestinal spirochaetosis in comparison with infections due to other enteropathogenic agents in a selected group of patients

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Objectives: Intestinal Spirochaetosis (IS) is a human large bowel infection due to Brachyspira aalborgi and B. pilosicoli associated with abdominal complaints (diarrhoea, pain, weight loss).
The prevalence of IS in the general population in comparison with other gastrointestinal infections was poorly investigated till now.
In our laboratory, we studied the occurrence of IS in a selected population with gastrointestinal complaints and/or potential risk factors for IS (immigration/travels from/through developing countries, HIV infection, male homosexuality) and compared the results with the occurrence of gastrointestinal infections due to viruses, bacteria other than spirochaetes and parasites.
Methods: In the period 2002–2007, clinical samples belonging to 269 patients with gastrointestinal disorders were subjected to microbiological and virological investigations in our laboratory to search for enteropathogenic agents: from 258 patients we received faecal samples, from 9 patients both faecal samples and colonic biopsies, and from 2 only colonic biopsies.
The research of intestinal spirochaetes was performed by a 16S rDNA Restriction Fragment Length Polymorphism-Polymerase Chain Reaction (RFLP-PCR) assay and PCR-positive samples were inoculated in a selective blood agar medium with spectinomycin and rifampin.
Enteropathogenic agents other than spirochaetes were searched according to standard procedures by conventional (microscopy and culture) and molecular methods.
Results: In 5 years 19 cases of IS (7%) were diagnosed by RFLP-PCR: 11 by B. aalborgi, 6 by B. pilosicoli and 2 mixed infections by both species. Ten of these patients were also infected by enteropathogenic agents other than spirochaetes.
The other patients were infected by intestinal parasites only (17.1%), gastroenteric viruses only (3.7%) or bacteria other than spirochaetes only (5.9%) whilst in a relevant group of individuals (66.1%) no enteropathogenic agents were detected.
Conclusions: Even if it refers to a selected population presenting gastrointestinal symptoms and risk factors for faecal-oral infections, the prevalence of IS reported in this study (7%) is of interest: in fact, our data suggest that IS may be more frequent than suspected in comparison with other gastrointestinal infections.
Therefore, we are confident that physicians should take IS into account when subjects with chronic abdominal discomfort present to their attention.

P921 Whole genome pyrosequencing of the epidemic multidrug-resistant *Acinetobacter baumannii* European clone II

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Objectives: the whole genomic DNA sequence was determined for an epidemic *Acinetobacter baumannii* strain (ACICU), belonging to the European clone II and carrying the plasmid-mediated blaOXA58 gene, implicated in carbapenem resistance. We compared the ACICU genome with that of *A. baumannii* ATCC 17978 and of *Acinetobacter baylyi* ADP-1, with the aim of identifying novel traits related to virulence, drug resistance and pathogenesis.
Methods: the genome was sequenced by high-density pyrosequencing and characterised for both genes coding and non coding. The genome was also analysed for lateral gene transfer.
Results: *A. baumannii* ACICU strain contains a single circular cromosome of 3,904,116 bp and two plasmids (ACICUp1 and ACICUp2) of 28,279 and 64,366 bp, respectively; the chromosome contains 3,758 genes. (3,670 CDSs, 64 tRNA genes 8 rRNA operons).
Genome comparison showed 86.4% of syntheny versus the reference *A. baumannii* ATCC 17978 strain and 14.81% versus *A. baylyi* ADP-1.
36 putative alien islands (pAs) are detected in the ACICU genome: 24 of them were previously described in the ATCC 17978 genome, 4 identified by the lateral gene transfer analysis in both ATCC and ACICU and 8 completely new, being absent in the ATCC 17978 genome
21 putative TonB-dependent outer membrane receptors were found in the ACICU genome, likely involved in iron uptake. ACICU has a conspicuous number of transporters belonging to different superfamilies, the number of which for unit length of genome (76.2) is much higher than that of ATCC 17978 (57.2) and ADP-1 (62.5), and similar to values reported for some pseudomonads.
Moreover, an antibiotic resistant island, AbaR2, was identified but it is shorter than the AbaR1 previously described in the multiresistant *A. baumannii* AYE strain.

Conclusion: the pathogenicity of *A. baumannii* is poorly understood and this organism is commonly regarded as a low-virulence bacterium, in spite of the severity of infections it causes. pAs in ACICU genome are partly divergent from those described in ATCC 17978, by acquisition of several interesting genes encoding membrane transporters, drug resistance and virulence trait for invasion and adherence. Four clusters of acquired genes were identified associated to putative phages. Since phage-related pathogenicity islands have been identified in the genome of important pathogens, the presence of unknown sequences poses the question of whether they could be involved in pathogenicity.

P922 Draft genome sequencing of two *Enterococcus faecium* strains by pyrosequencing technology reveals niche-specific gene acquisition

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Objectives: *Enterococcus faecium* has recently become an important cause of nosocomial infections. The rapid rise in *E. faecium* infections can be explained by the acquisition of antibiotic resistance mechanisms combined with the evolution of a hospital-associated *E. faecium* clade, termed Clonal Complex 17 (CC17). To reveal the genetic diversity in *E. faecium* strains from different environmental niches, we sequenced the genomes of two *E. faecium* strains from different clonal complexes (CC22 and CC17, respectively) using pyrosequencing technology.
Methods: The strains studied were E1071 (CC22, a vancomycin-resistant isolate that was picked up during routine hospital surveillance) and E1162 (CC17, a vancomycin-sensitive bloodstream isolate). The genomes of these two *E. faecium* strains were sequenced by Keygene (Wageningen, The Netherlands) using the GS FLX sequencer (454 Life Sciences, Branford CT, USA), with 20-fold coverage of the genome. Sequences were assembled using Newbler Assembler (454 Life Sciences). The draft genomes were annotated using the TIGR Annotation Engine and the encoded proteins were compared using PROMPT (Protein Mapping and Comparison Tool; http://webclu.bio.wzw.tum.de/prompt).
Results: The total number of sequence that could be assembled into contigs was 2.7 Mb for both strains, suggesting all but complete coverage of the genome. Automated and manually curated annotation of the draft genome sequences resulted in the prediction of 2716 and 2697 proteins for E1071 and E1162, respectively. More than 2200 proteins were more than 95% identical on the amino acid level between the two strains. Approximately 10% of the proteins were unique to each strain.
Using PROMPT the predicted proteins from the genomes of these two strains and the publicly available *E. faecium* DO strain (CC17) were compared. This revealed that the CC17 strains had genomic islands with functions in sugar uptake and metabolism that were absent in E1071. Two (partial) prophages were also specific for the CC17 strains. The presence of the TcrB copper resistance system and a specific point mutation in the vanX gene in strain E1071 strongly suggest that this strain originates from pigs.
Conclusion: Rapid draft genome sequencing has revealed the acquisition of several niche-specific genes in *E. faecium* and identified several genes and genetic elements that may be unique for CC17. The genome sequences contribute to ongoing functional studies into *E. faecium* in our laboratory.

P923 Identification of a novel genomic island specific for *Enterococcus faecium* CC17 by using the deltarho-Web model

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Objectives: Most hospital outbreak and invasive *Enterococcus faecium* belong to a single clonal lineage, complex 17 (CC17). We hypothesise that acquisition of genes, involved in antibiotic resistance, metabolic pathways, and virulence, contributes to the ecological success of CC17. So far, antibiotic resistance genes and the enterococcal surface protein gene, esp, which is contained on a putative pathogenicity island and plays an important role in biofilm formation, are documented CC17-specific determinants. To identify more genes, which contribute to the success of CC17, we used the deltarho-Web model (http://deltarho.amc.uva.nl). This model analyses the dinucleotide composition of a complete genome, which allows the identification of horizontally transferred genes, recognised by their anomalous dinucleotide frequency (DF).
Methods: A complete genome sequence was designed from the only sequenced strain, *E. faecium* DO, a CC17 isolate. Because this strain is sequenced partially and the order of the contigs is not known, we created a so-called complete genome sequence by randomly concatenating all contigs larger than 2022 bp, which was submitted to the model. PCR and dot blot hybridisation (DBH) were performed to determine presence and absence of genes located in regions with anomalous DF in 41 CC17 isolates and 95 non-CC17 isolates. Expression of CC17 specific genes at mRNA level was determined by RT-PCR.
Results: The model identified 5 regions with anomalous DF. PCR and DBH showed that 1 of these regions is CC17 specific and consist of a genomic island (GI) of 8 genes. Forty of the 41 CC17 isolates (97.56%) harbour this GI and only 5 of the 95 non-CC17 isolates (5.26%). The GI genes putatively encode 2 sugar transporters, 2 glycosyl hydrolases, an extracellular sugar binding protein, 2 proteins with unknown function, and a transcriptional regulator. At both sides of the GI inverted and direct repeats were found and one end is flanked by an integrase gene, indicating horizontal transfer. RT-PCR showed expression of all 8 genes.
Conclusion: By using the deltarho-Web model, a GI specific for CC17 was found. This illustrates that detection of anomalous DF using this model is a good approach for identifying acquired genes in *E. faecium*. We hypothesise that ST-17, the presumed founder of CC17, acquired this GI, tentatively encoding a novel metabolic pathway, as an adaptive mechanism providing CC17 a selective advantage in hospitalised patients.

P924 Presence of pRI1; a small cryptic mobilisable plasmid isolated from *Enterococcus faecium* of human and animal origin

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Objectives: This study focused on the molecular characterisation of a small cryptic mobilizable plasmid (6038 bp) sequenced from an *E. faecium* 9631160–1 of poultry origin. Presence of the pRI1 replication initiation gene (rep) was analysed in a panel of *E. faecium* isolates from human and animal origin in different European countries.
Methods: A plasmid replicon was cloned in vector pVA891. The insert was sequenced and primers were designed to complete the entire DNA molecule. 159 isolates were screened by PCR for the presence of the pRI1 rep. Of those, 40 were vancomycin resistant *E. faecium* (VREF) of broiler origin from the UK, 92 were of animal origin (broiler=41, pig=51) from Denmark (DK) and 27 were VREF isolated from hospitalised patients, 7 from Germany (DE) and 20 belonging to the CC17 and collected across Europe. Transfer of the pRI1 was assessed by selecting transconjugants in media with vancomycin or streptogramin.
Results: Sequence analysis of pRI1 revealed 8 open reading frames. Based on sequence similarity at DNA and protein level, pRI1 contained a rep highly identical to the repA from the pEFNP1 plasmid from *E. faecium* N15. A mob gene was also identified whose putative function could be the mobilisation of the plasmid. pRI1 harboured a cluster of 4 coding sequences (CDS). CDS1 translated into a truncated EfaeDRAFT_2604. CDS2, CDS3 and CDS4 coded for 3 hypothetical proteins, EfaeDRAFT_2603, EfaeDRAFT_2602 and EfaeDRAFT_2601 respectively. The 4 CDS exhibited 100% homology to those described in contig658 (from nt 8940 to 10196) of *E. faecium* DO. In addition, an IS with 100% homology to ISEfa4 described in a vanD-type VREF 10/96A was also identified. Of 159 isolates tested for presence of pRI1 rep, 60 produced an amplicon. Of those, 27/40 were from UK, 22/41 and 2/51 of broiler and pig origin from DK, 5/7 from DE and 4/20 belonged to the CC17. Conjugation experiments verified transfer of the pRI1 together with conjugative plasmids harbouring resistances to vancomycin and streptogramin.
Conclusions: The genetic composition of pRI1 illustrates the plasticity of the enterococcal plasmids and the "mosaic" arrangements of the

different genes assembled into one mobile genetic element (MGE). The presence of pRI1 in enterococcal isolates geographically separated and from different origin demonstrates the ability of enterococci to acquire and transfer MGE, emphasising the need for further studies to reveal the meaning and role that they play in nature.

Current methods for typing MRSA and MSSA

P925 Comparison of the DiversiLab System and pulsed-field gel electrophoresis for typing meticillin-resistant *Staphylococcus aureus* isolates

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Objectives: Typing of meticillin-resistant *Staphylococcus aureus* (MRSA) isolates is difficult for clinical microbiology laboratories to perform. Pulsed-field gel electrophoresis (PFGE) is time consuming and often difficult to interpret for inexperienced users. Thus, we examined the ability of the Bacterial Barcodes DiversiLab system (DL) to distinguish among USA PFGE types and to correctly differentiate outbreak-related MRSA from sporadic isolates collected from several U.S. hospitals.
Methods: 105 isolates of MRSA of PFGE types USA100–1100 (plus the Brazilian clone) from the ICARE and CDC strain collections were typed using the DL system. DL strain relatedness was defined as a minimum of >95% similarity with up to 1 band difference. USA types were defined using >80% similarity. In addition, 4 unique sets consisting of 6 MRSA isolates each (where 5 isolates were indistinguishable by DL and 1 was an unrelated DL type), each from separate outbreaks in 3 U.S. hospitals, were typed by PFGE. Tenover criteria were used for PFGE outbreak analysis.
Results: DL separated the 105 MRSA isolates of known USA types into 13 clusters and 6 singletons. DL grouped USA100, 200, 600, and 1100 isolates into unique clusters. Multi-locus Sequence Type 8 isolates (USA300, 500 and Brazilian clone) often clustered together at >95% similarity in the DL dendrogram, although USA300 and 500 DL patterns showed a consistent 1 band difference. USA400 and 700 isolates also clustered together but had distinguishable DL patterns. Among the hospital outbreak clusters, PFGE and DL both identified the same "unrelated" organism in all 4 sets; however, PFGE showed more pattern diversity than did DL.
Conclusion: DL is less discriminatory than PFGE, although it tends to cluster isolates of the same USA type together. DL can be used to identify USA types only by direct comparison of DL patterns with a known USA type strain. Although the DL patterns of the outbreak strains were indistinguishable, the PFGE patterns of 3 of 4 sets reflected greater pattern diversity. Thus, DL is useful for screening for potential outbreaks of MRSA, but additional typing may be necessary to confirm strain relatedness and specific USA types.

P926 Comparison of an automated repetitive sequence-based PCR typing technique to pulsed-field gelelectrophoresis and multi-locus sequence typing for characterisation of epidemic meticillin-resistant *Staphylococcus aureus* strains

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Objective: the DiversiLab™ system, which employs repetitive sequence-based PCR (rep-PCR) to genetically characterise microorganisms (prokaryotes and eukaryotes), was evealuated as a molecular typing tool for meticillin-resistant *Staphylococcus aureus* (MRSA) strains.
Methods: we have analysed a representative selection of the HARMONY collection, composed of MRSA strains (n = 33) from 11 European countries. DiversiLab results were compared to those assessed by pulsed-field gelelectrophoresis (PFGE) and clonal designations based on multilocus sequence typing (MLST).
Results: the level of discrimination was determined by application of Simpson's index of diversity, resulting in indices of 90.5%, 87.7% and 86.0% for PFGE, MLST and rep-PCR respectively. A high level of concordance in classification of the MRSA strains was obtained using each method. MLST and rep-PCR distinguished 15 and 17 genetically related groups, as PFGE differentiated the collection into 28 pulsetypes.
Conclusion: compared to both other typing techniques, the performance of the DiversiLab is good with respect to labour intensity and rapidity. This system may be useful for long-term epidemiological studies, but lack the discrimination needed for outbreak analysis.

P927 Epidemiological typing of meticillin-resistant *Staphylococcus aureus* strains by FT-IR

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Meticillin-resistant *Staphylococcus aureus* (MRSA) is widely known as the most significant nosocomial pathogen due to the burden of disease it causes, as well as to the evolution and global spread of multidrug-resistant clones.
Objective: The goal of the present study is to evaluate the Fourier transform infrared (FTIR) spectroscopy as a rapid, accurate and cost-effective method to characterise and discriminate between MRSA strains responsible for nosocomial infections.
Methods: MRSA strains were studied by Pulse Field Gel Electrophorese (PFGE – enzyme SmaI) and rep-PCR, two molecular methodologies that provide the genomic fingerprint with high discriminatory power. Although time consuming and labour intensive PFGE is accepted as the gold standard for molecular typing. MRSA strains were also studied by FTIR assess the overall molecular composition of microbial cells in a non destructive manner, giving the "whole organism fingerprinting". The collected spectral data were analysed using a wide range of chemometrics techniques to discriminate closely related strains.
Results: MRSA were separated in distinct clones by PFGE, rep-PCR and FTIR. Analysis of SmaI macrorestriction profiles of the MRSA clinical isolates revealed five major clusters. The rep-PCR allowed grouping strains within various degrees of genotypic relatedness leading to the identification of bacterial clones. All the strains were evaluated by FTIR spectroscopy as well. Different pre-processing methods were analysed in order to reduce spectral variability. Unsupervised and supervised classification methods were employed to cluster the different MRSA clones based on similarity of their FTIR spectra. Our results demonstrated concordance with PFGE and rep-PCR methods.
Conclusion: FTIR spectroscopy is a promising tool for epidemiological typing of MRSA, providing quantitative information about the total biochemical composition of the intact cell reflected in the infrared spectra. FTIR is a simple, non-destructive, without use of any reagent and rapid to perform thus has the potential for an alternative method for MRSA epidemiological typing. In case of nosocomial outbreak FTIR enables a fast identification of MRSA clones responsible for hospital infection and a rapid implementation of controlling measures leading to a decreasing of new cases of nosocomial infection due to the same bacterial clone, saving suffering and money.

P928 Spa typing is an excellent method for surveillance of MRSA

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Objectives: Pulsed-field gel electrophoresis (PFGE) is considered gold standard for typing of *Staphylococcus aureus*, but the method is afflicted with problems. Simple objective molecular methods, like spa typing, can therefore considerably simplify epidemiological studies of *S. aureus*. In this study we compare spa typing to PFGE, related to epidemiological data in a low endemic setting, and show that spa typing is an excellent method for surveillance of transient outbreaks of meticillin-resistant *S. aureus* (MRSA).
Methods: All of the MRSA (n = 304) isolated in the county of Jonkoping from January 2003 through July 2006 were analysed by spa typing and PFGE. In addition, Panton-Valentine leukocidin (PVL) toxin presence

was investigated using a PCR based method. Epidemiological data was collected and 12 outbreaks were identified, including 68 isolates.

Results: We identified 24 different spa types and 28 different PFGE patterns in 20 PFGE types. Isolates within three of 12 outbreaks exhibited several related but distinguishable PFGE patterns. In seven of the outbreaks all of the isolates were of a single PFGE type (SE97–3). All of the isolates within an outbreak were of the same spa type. Three outbreaks contained isolates of spa type t015 of which all belonged to PFGE type SE97–3 and two outbreaks consisted of isolates of spa type t008.

Isolates carrying the PVL toxin gene, occurring in outbreaks, were all community-associated (Table 1).

Table 1: Description of the MRSA outbreaks in the county of Jönköping, 2003–2006

Outbreak	Number and association in outbreaks	*spa* type	PFGE pattern	PVL
1	2, neighbours	t202	SE03-9	+
2	3, family	t015	SE97-3f	–
3	8, associated to retirement home	t630	SE97-3, -3t, -3v	–
4	11, associated to retirement home	t008	SE00–11a, -11d	–
5	12, associated to retirement home	t050	SE97-3af, -3, -3d	–
6	3, hospital	t015	SE97-3	–
7	2, family	t008	SE00-7d	+
8	15, associated to retirement home	t669	SE97-3af	–
9	3, associated to retirement home	t223	SE04-4a	–
10	3, hospital	t993	SE97-3f	–
11	3, family	t015	SE97-3a	–
12	3, family	t1096	SE01-3	+

Conclusion: All isolates within an epidemiological well characterised outbreak were of the same spa type, revealing better concordance to epidemiological data than PFGE as three outbreaks included isolates of PFGE patterns with minor differences, splitting well defined outbreaks into more than one. Furthermore spa typing is superior to PFGE in routine microbiological laboratories due to its objectivity, short turn-over time, ease of use, and possibility of interlaboratory comparability. spa typing must therefore be considered a very useful method for epidemiological investigations of MRSA in low prevalence areas.

P929 Molecular characterisation of *Staphylococcus aureus* dialysis associated bacteraemia isolates. Developing microarray analysis towards future rapid typing applications

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Objectives: *Staphylococcus aureus* is an important pathogen in healthcare associated infection. Within the haemodialysis setting it is responsible for high levels of morbidity and mortality and associated with access related bacteraemia. A particular problem with in any healthcare setting is defining preventable transmission and recurrence of clinical infection. To aid this definition we need timely locally applicable typing systems that are technically and financially suitable for everyday use. Our objective was to use well characterised isolates from the dialysis associated bacteraemia setting as a platform towards developing a clinically relevant rapid typing system which would eventually be of use within a routine diagnostic laboratory.

Methods: Thirty-nine consecutive clinically relevant renal *S. aureus* bacteraemia isolates collected between July 2002 and March 2003 were characterised by antibiogram, phage typing, pulsed-field gel electrophoresis (PFGE) and multi-locus sequence typing (MLST). Meticillin resistance was detected in 46% of isolates and defined by phenotypic and molecular methods. A defined representative subset of isolates were characterised using a staphylococcal microarray. The combined approach was analysed using Bionumeric software.

Results: Phage typing followed by PFGE revealed eight patterns, clustering into 2 major types, EMRSA-15 and EMRSA-16. MLST showed that the MRSA isolates belong to ST22 and ST36. Isolates that were identical by MLST had either identical or similar PFGE fragment patterns. Microarray analysis allowed the definition of 'core' and 'accessory' genomes and the identification of potential amplification targets for use in a relevant and rapid typing scheme. Accessory genes were defined by approximately 23% of 4016 staphylococcal features on the array and is supported by current literature in this area. Bionumeric software is displays the level of congruence between PFGE and microarray analysis.

Conclusion: EMRSA-15 and 16 were identified as major groups within which to define future subtyping targets. Suitable gene targets for discrimination are identified and can be defined and developed in this way. This is a realistic approach towards developing novel technology based typing systems that could be used in future diagnostic settings.

P930 Investigation of epidemiological links between patients harbouring identical, non-predominant MRSA genotypes as defined by double-locus sequence typing

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Background: Molecular epidemiology postulates that 2 isolates belong to the same chain of transmission if they are similar according to a highly discriminatory molecular typing method. This has been demonstrated in outbreaks, but was never studied in endemic situations. Epidemiological links cannot be established between isolates of meticillin-resistant *Staphylococcus aureus* (MRSA) that belong to endemic predominant genotypes. In contrast, small clusters of identical genotypes, some of which are subtypes of predominant genotypes, could be studied for epidemiological tracking.

Objective: To investigate epidemiological links between patients harbouring MRSA genotypes that belong to non-predominant typing clusters at a regional level.

Methods: We recently showed that double-locus sequence typing (DLST), a new typing strategy for MRSA that uses the repeat sequences of clfB and spa genes, was reproducible and discriminant enough to make this method a candidate of choice for epidemiological analyses. Our laboratory centralises the majority of the MRSA isolates from Western Switzerland. All nonrepetitive isolates received between 2005 and 2006 were typed by DLST.

Results: 1658 MRSA isolates were typed. 1118 (67%) belonged to 4 predominant genotypes, 216 (13%) to 54 small clusters of 2–12 patients, and 324 (20%) were unique. Strong epidemiological links (simultaneous hospitalisation in the same ward/hospital/long term care facility, or member of the same family) were found in 22/54 (41%) small clusters of non-predominant genotypes, totalising 60/216 patients (28%). In addition, weaker epidemiological links (hospitalisation during the same year in the same ward/hospital/long term care facility, or same place of residence) were found in 41/216 patients (19%). In the remaining 115/216 (53%) patients harbouring identical non-predominant genotypes, no epidemiological link could be detected.

Discussion: In our setting of patients harbouring non-predominant MRSA genotypes, epidemiological data could not explain the chain of transmission behind all isolates of the same genotype. One hypothesis is that our surveillance system was not able to detect the missing links. Another hypothesis is that some genotypes are stable over time, so that, as for predominant genotypes, epidemiological links can no longer be found.

Conclusions: Epidemiological tracking of MRSA isolates at a regional level cannot be inferred from molecular typing alone.

P931 Double locus sequence typing: a high through-put molecular typing method for meticillin-resistant *Staphylococcus aureus*

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Background: Molecular typing is required for the surveillance of meticillin resistant *Staphylococcus aureus*. We recently showed that

DLST, a new typing strategy for MRSA was reproducible, as discriminant as PFGE and gave unambiguous results (JCM 2007, 45:54–62). Rapidity, accessibility, ease of use, cost and amenability to construct an electronic database, are other important criteria of convenience for longitudinal comparison of large numbers of isolates (CMI 13, suppl. 3; 2007).

Objective: To evaluate DLST according to convenience criteria for the surveillance of MRSA at a regional level.

Material and Methods: DLST is based on single strand sequencing of partial repeat sequences of genes clfB and spa. All procedures and reactions were adapted to function in 96 well format. Our laboratory centralised the majority of the MRSA isolates from Western Switzerland. All isolates received between 2005 and June 2007 were typed by DLST.

Results: The laboratory isolated and received 1668 isolates during the study period. Typing of one plate (96 isolates) was performed in a weeks time (including bench work, sequence analysis, and incorporation into a Bionumerics database); all isolates were processed within 18 weeks. Reinjection was necessary for 15.2% of clfB and 5.7% of spa sequencing reactions, and the DNA of 1.3% of all isolates had to be extracted again. The cost of consumables was calculated to be Euro 9.-/isolates, (versus Euro 2.40 with PFGE), and the staffing cost Euro 13.–/isolates (versus 26.– with PFGE).

Discussion: Cost efficiency was achieved by performing DLST in 96 well plates. In a weeks time, 96 isolates could be typed, whereas only 48 isolates could be typed by PFGE using a rapid protocol (JCM 1992; 30:577–80). DLST was cheaper than PFGE, and accessible to all laboratories with sequencing facilities. The low percentage of isolates from which DNA had to be extracted a second time indicated a robust protocol. Using an improved polymerase for PCR, the percentage of clfB sequence reinjection was considerably reduced. The major advantage of DLST is that genotypes can be unambiguously assigned, which allows to incorporate results in an electronics database.

Conclusions: The use of DLST proved to be a convenient method for longitudinal surveillance of MRSA genotypes at a regional level.

P932 Species identification and simultaneous detection of rifampicin resistance in staphylococci using rpoB sequencing

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Objectives: In recent years, coagulase-negative staphylococci (CoNS) have been recognised as causative agents of various infections, especially in immunocompromised patients and related to implanted foreign body materials. A major problem is to distinguish clinically significant CoNS strains from those representing contamination. Therefore rapid, highly sensitive and specific methods are needed for species identification and further typing. Foreign body infections caused by CoNS are difficult to treat since CoNS have the ability to produce biofilm. β-lactams and glycopeptides lack activity against biofilm producing and stationary phase bacteria. In contrast, rifampicin has the ability to penetrate biofilm. However, resistance to rifampicin may rapidly emerge and, accordingly, monotherapy should be avoided.

The aim was to develop and evaluate a rapid and objective method, based on rpoB sequencing, for species identification and simultaneous detection of rifampicin resistance in staphylococci.

Methods: Fifty-one isolates, representing 17 different *Staphylococcus* species, were included for species identification, and isolates (n = 30) from eight patients, obtained pre- and post-treatment with rifampicin, were included for detection of rifampicin resistance.

A real-time PCR followed by sequencing was developed for a 1052 bp segment of the rpoB gene. The sequences were analysed using BioEdit software v. 7.0.5.3 and submitted to GenBank for species identification. In case of discrepancies between the initial phenotypic species identification (using ID32Staph) and rpoB sequencing, 16S rRNA gene sequencing was used.

Results: Forty-nine (96%) of the 51 isolates were possible to species identify using rpoB sequencing and the two remaining isolates were not staphylococci according to the discrepancy analysis. Comparing the results of rpoB sequencing with the phenotypic identification revealed that eight (16%) of the 49 isolates differed regarding identified species. Each isolate resistant to rifampicin from the eight patients displayed identical rpoB sequence as its corresponding rifampicin susceptible isolate except for one (in six patients) or two (n = 3) nonsynonymous single nucleotide polymorphisms (SNPs), or an insertion of one codon, which has not previously been described (n = 3).

Conclusion: Sequencing of the rpoB gene is a rapid, objective and accurate method for species identification and simultaneous detection of rifampicin resistance in staphylococci.

P933 Application of the (GTG)5-PCR fingerprinting for rapid identification of staphylococcal species occurring in humans

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Objectives: Staphylococci are natural inhabitants of skin, glands and mucous membranes of mammals and birds and play very important role in human infections as well. Development and evaluation of effective and reliable identification tools is needed for routine and rapid identification of increasing number of *Staphylococcus* spp. occurring in human clinical material.

Method: A group of 68 type and reference staphylococcal strains representing 22 taxa of human associated staphylococci was analysed by rep-PCR fingerprinting with the (GTG)5 primer. The clinical isolates as well as strains isolated from healthy hosts and environmental samples were tested.

Results: All tested strains were typeable by the (GTG)5-PCR producing DNA fragments ranging from 200 bp to 4500 bp. Majority of the tested species revealed species-specific fingerprints, moreover *Staphylococcus capitis* ssp. *capitis*, *S. capitis* ssp. *ureolyticus* and *Staphylococcus cohnii* ssp. *cohnii* and *S. cohnii* ssp. *urealyticum* were differentiated at the subspecies level. In contrast, *Staphylococcus hominis* subspecies were not clearly distinguished. *Staphylococcus sciuri* ssp. *sciuri*, *Staphylococcus intermedius* and *Staphylococcus cohnii* ssp. *urealyticum* strains revealed two different fingerprint clusters each. The highest intraspecies similarity showed *Staphylococcus warneri* (88.95%) and *Staphylococcus auricularis* (87%) strains in this study.

Conclusion: The obtained results imply that rep-PCR fingerprinting with the (GTG)5 primer is suitable method for reliable and rapid identification of the majority of staphylococcal species occurring in humans.

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P934 Evaluation of MLST for *Staphylococcus aureus* typing using labelled-primers chemistry

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Objective: Staphylococcal typing is important for studying nosocomial and community outbreaks, defining the relationship between isolates. Multilocus Sequence Typing (MLST) relates staphylococci on the basis of the nucleotide sequences of ~450 bp internal fragments of seven conserved housekeeping genes (arcC, aroE, glpF, gmk, pta, tpi, yqiL). For each gene fragment, the different sequences are assigned as distinct alleles at each of the seven housekeeping loci (the allelic profile or sequence type, ST). A major advantage of MLST is the ability to compare results obtained by different studies via Internet, in freely accessible databases. The purpose of the present study was the evaluation of MLST using the labeled-primers chemistry.

Methods: Five representative MRSA were selected according to their phenotypic characteristics, SCCmec and agr types, PFGE, and the presence of toxin genes. These strains were also previously characterised by MLST using the BigDye Terminator chemistry (BigDye Terminator v3.1 cycle sequencing Kit, Appliedbiosystems) and the ABI 3730 XL Capillary Sequencing (Appliedbiosystems) device. Bacterial cultures in Brain Heart Infusion broth (BHI), after overnight incubation at 37°C, were used for DNA extraction applying the phenol:chloroform method. The seven housekeeping genes were first amplified by PCR. PCR products were then purified and sequenced bi-directionally by

using labeled-primers chemistry (Cy5.5 for the forward and Cy5.0 for reverse primers) and the OpenGene DNA Sequencing System (Visible Genetics, Inc. Toronto, Ontario, Canada). PCR conditions were optimised in experiments applying gradient of primer concentrations.
Results: Optimised final concentration of primers was achieved at 0.1microM for the downstream and 0.15microM for the upstream in each reaction tube. Results from both MLST techniques concerning the allelic profiles, the Sequence Types (ST) and the Clonal Complexes (CC) agreed and no variations were detected.
Conclusion: The adaptation of MLST using the labeled-primers chemistry and vertical electrophoresis, which is for the first time reported, gave reproducible results, allowing its application in laboratories that do not possess a capillary sequencing device.

P935 Molecular characterisation of meticillin-resistant *Staphylococcus aureus* nasal isolates from patients with uncomplicated skin infections

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Objectives: In order to better understand the clonal composition and epidemiology of meticillin-resistant *Staphylococcus aureus* (MRSA) from nasal carriage, we molecularly characterised all nasal MRSA isolates recovered in 2004–2005 from patients with uncomplicated skin infections enrolled in Phase III clinical trials of retapamulin, a new topical antibiotic agent.
Methods: Nasal swab samples were obtained from patients with uncomplicated skin infections enrolled in five global phase III clinical trials. All nasal MRSA isolates were examined by pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), Staphylococcal cassette chromosome (SCCmec) typing, and were tested for the presence of Panton-Valentine leukocidin (PVL) genes. Nasal MRSA isolates were recovered from patients in 7 different countries (Costa Rica, France, Germany, India, Peru, South Africa, and the United States) but the vast majority were recovered from patients in the United States.
Results: A total of 105 MRSA isolates were recovered from nasal cultures at baseline and/or at follow up visits from 62 patients. Of the 54 baseline MRSA isolates, 23 (42.6%) were determined to be PFGE type USA300, PVL-positive and contained type IV SCCmec and of those 20 were also MLST type 8. Except for one isolate recovered from a patient in Costa Rica, the vast majority (19/20;95%) were recovered from patients in the United States. The remaining 31 MRSA isolates were distributed among 4 other recognised MLST/PFGE genotypes or were not matched to any PFGE types currently in the CDC database. Of the 30 patients who had a nasal MRSA isolate recovered from more than one follow up visit, 19 (63%) had the same PFGE type at each visit.
Conclusions: This study demonstrates that PVL-positive, USA300 MRSA was carried in the anterior nares of patients with uncomplicated skin infections and was found to be the predominant type collected from the baseline nasal samples of patients in the United States. The majority of patients who had MRSA isolated from their anterior nares at two or more visits maintained carriage of the same PFGE type. Both of these findings have significant implications for epidemiologic investigations and for planning future interventional studies.

P936 Determining evolutionary lineages of *Staphylococcus aureus* using variable number tandem repeats

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Objectives: The aim of the study was to determine the ability of variable number tandem repeat typing to define evolutionary lineages using a well characterised global collection of MSSA and MRSA isolates.
Methods: Ninety two isolates of *S. aureus* from diverse origins were included in the study. The isolates consisted of 41 MRSA (SCCmec types I to IV) and 51 MSSA isolates, as well as examples of isolates from the five major global clonal complexes. All isolates were typed using three sequence based typing methods, MLST, spa and sas typing and using 7 previously described variable number tandem repeats termed staphylococcal interspersed repeat units (SIRU). The numbers of repeats at each locus were determined and a 7 digit profile generated. All of the epidemiological typing data was analysed using Bionumerics and SIRU data analysed using Euclidian coefficient. Simpson's index of diversity was used to calculate the index of discrimination of all the typing techniques.
Results: All isolates were typeable by all four typing methods, and gave 100% reproducibility. SIRU typing provided the greatest level of discrimination (D=0.997 CI 0.995, 1.000), significantly greater ($P<0.05$) than that provided by the most discriminatory sequence based method, spa typing (D=0.9847, CI 0.975 to 0.994). Both spa and sas typing gave a higher index of discrimination than MLST (0.965 versus 0.939 respectively).
Cluster analysis of the SIRU profiles demonstrated concordance with the MLST data, whilst providing a greater degree of diversity. Isolates that are diverse by MLST (greater than two loci different) were not clustered together by SIRU. SIRU typing arranged the isolates into five main groups, clustering together isolates from CC30, CC22, CC1, and CC5 into distinct complexes, while the majority of isolates from CC8 were clustered together. Singleton strains, those unrelated to the major clonal complexes, were diversely spread.
Conclusion: This study uses isolates that have been previously used to define the evolution of MRSA and clustering analysis demonstrates that SIRU has excellent concordance with previously used sequence based typing methods. SIRU provides a greater degree of discrimination than MLST allowing a more detailed insight into the evolution of individual clonal complexes, whilst still retaining the overall population structure exhibited in the clonal complexes defined by MLST.

P937 Variable number tandem repeat assay for typing meticillin-resistant *Staphylococcus aureus*

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In the work with pathogen microorganisms, the knowledge of their DNA fingerprints is necessary for the delineation of outbreaks of infections disease and for the global tracking of them. For these reasons, on the last decades an intense search of methods to improve this way has taken place. Variable Number Tandem Repeat Assay (MLVA) was described for the first time in 1995 as a new typing method for *Haemophilus influenzae*. Later, in 2003 Sabat et al. introduced it for the study of *Staphylococcus aureus*. However, the usefulness of this technique is still an opened question.
Objectives: The aim of our study has been to determine the utility of MLVA for typing *S. aureus* isolates and to compare the results obtained with those generated by means of the Pulsed-Field Gel Electrophoresis (PFGE).
Methods: A multiplex PCR assay, optimised by us, containing primers to simultaneously amplify clfA, clfB, sdrCDE, spa and sspa loci, and PFGE macrorrestriction patterns analysis were applied to a group of meticillin-resistant *S. aureus* (MRSA), collected since 2002 to 2006. The results obtained by both approaches were compared by means of the power of discrimination of each of them.
Results: All MRSA isolates were typeable by MLVA, being grouped in 23 types, according to the criteria established by Sabat et al. (2003), whereas the PFGE assay distinguished 17 types according to the criteria established by Tennover et al. (1995). As a general rule, within each pulsed-field type, a majority MLVA pattern and others that had one band change with this were noticed.
Conclusion: MLVA is a reliable technique, with a high power to discriminate MRSA isolates. This capacity can be used to perform a local characterisation of MRSA collections. MLVA patterns seem to belong with PFGE types, whenever we consider subtypes (one band different of the majority pattern) inside MLVA types.

Diagnostic serology

P938 Multicentre evaluation of the Toxo IgG assay on the Family of Access® immunoassay systems from Beckman Coulter

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Background: *Toxoplasma gondii* is a protozoan capable of infecting many species of mammals and birds. Human infection is common worldwide, although prevalence varies with subject age and geographic location. Infections in adults and children past the neonatal stage is usually unapparent. Primary infection acquired just before and during pregnancy may lead to fetal death or serious congenital abnormalities. Serological confirmation of antibodies to *Toxoplasma* is indicative of exposure to the parasite and is the principal means of diagnosis and follow-up.
The Access® Toxo IgG assay is based on paramagnetic particle, solid phase technology and chemiluminescent signal detection. The Access Toxo IgG assay has been restandardised to the WHO 3rd international standard TOXM.
Objectives and Methodology: Access Toxo IgG assay reproducibility was evaluated at three centres. Reproducibility was determined with nine samples with varying degrees of reactivity and two controls, analysing five replicates of each sample for seven days. Concordance (percent agreement) with the Abbott AxSYM assay was evaluated at two sites. Two hundred and seventy nine de-identified residual samples from pregnant subjects were tested as well as four hundred and seventy one non-selected samples from male and female subjects who had Toxo IgG testing ordered. The platform utilised at all centres was the Access 2 Immunoassay System. Similar performance has been demonstrated on the UniCel® DxI 800 Access Immunoassay System.
Results: At the cut-off (10.5 IU/mL) within site total CV% ranged from 8% to 10%. The overall agreement of the Access Toxo IgG assay to the AxSYM Toxo IgG assay was 97.3% (95% CI, 95.9% to 98.4%). The relative sensitivity was 98.0% (95.9% to 99.2%) and the relative specificity was 99.5% (98.1% to 99.9%).
Conclusion: The Access Toxo IgG assay provides excellent concordance to the comparison method to aid in the diagnosis of *Toxoplasma gondii* infection and the determination of protective levels of antibodies in pregnant women and the general population with the advantage of a rapid, automated random-access immunoassay system.

P939 Analytical performances of the new Access® Toxo IgG assay

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Objectives: In order to be aligned with international standardisation recommendations, Beckman Coulter has restandardised the Access® Toxo IgG assay to the WHO 3rd International Standard (TOXM). The purpose of this study was to evaluate the analytical sensitivity including limit of blank (LoB), limit of detection (LoD), limit of quantification (LoQ), as well as linearity, precision and analytical specificity of the new Access Toxo IgG assay.
Methods: The analytical sensitivity was determined according to the CLSI EP17-A procedure. LoB and LoD were defined with four negative and low level samples tested 60 times. LoQ, determined with samples with known expected *Toxoplasma* IgG concentration, was determined over five days with one run/day and five replicates per run. Linearity was studied with *Toxoplasma* IgG high positive samples diluted from 1:1.5 to 1:32. The precision studies used negative, low, medium and high positive samples and included intra- and interassay determinations or studies as well as inter-lot studies. Analytical specificity was determined by testing 310 samples obtained from patients with specific disease conditions.
Results: The Access Toxo IgG assay displays a LoB, LoD and LoQ of 0.10 IU/mL, 0.40 IU/mL and 3.2 IU/mL, respectively. The linear regression slope for linearity study was 1.07 with a total mean recovery percentage of 99.9%. The precision studies demonstrated a CV below 6%, 11% and 11% for intra-assay, inter-assay and inter-lot, respectively. Among the 310 samples tested for the analytical specificity, 303 were found negative with the new Access Toxo IgG assay.
Conclusion: The new Access Toxo IgG assay provides excellent performance, with the advantages of a rapid, automated random access immunoassay system.

P940 Comparative studies of the new Access® Toxo IgG assay

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Objectives: A new quantitative Access Toxo IgG assay, restandardised by Beckman Coulter to the WHO 3rd International Standard (TOXM), was studied in order to determine concordance between the new assay and the AxSYM** Toxo IgG assay. Relative specificity and sensitivity were calculated within the tested populations as compared to a reference method.
Methods: The concordance studies were performed with samples from non-selected blood donors (n = 700), non-selected hospitalised patients (n = 3824) and non-selected pregnant women (n = 358). The comparative device used for these studies was the Abbott AxSYM Toxo IgG assay and a percentage of agreement between the methods was calculated. Specificity and sensitivity were calculated based on a final interpretation of the results (defined as a reference method) obtained with the current Access Toxo IgG, the AxSYM Toxo IgG and the bioMérieux VIDAS Toxo IgG assays.
Results:

- Blood donors: The agreement between the new Access Toxo IgG and the AxSYM Toxo IgG assays was 97.57% (683/700). According to the reference methods, the new Access Toxo IgG assay displayed specificity and sensitivity of 99.53% and 99.37%, respectively.
- Non selected hospitalised patients: The agreement between the new Toxo IgG and the AxSYM Toxo IgG assays was 97.64% (1781/1824). According to the reference methods, the new Access Toxo IgG assay displayed a relative specificity and sensitivity of 98.89% and 99.70%, respectively.
- Pregnant women: The agreement between the new Toxo IgG and the AxSYM Toxo IgG assays was 98.92% (552/558). According to a reference method, the new Access Toxo IgG assay displayed specificity and sensitivity of 100% and 100%, respectively.

Conclusion: The new Access Toxo IgG assay provides good agreement in comparison with the AxSYM Toxo IgG assay. The new Access Toxo IgG assay displays excellent relative specificity and sensitivity results compared to reference Toxo IgG methods. The new Access Toxo IgG assay can be used either with the Access® Immunoassay System or with the high-throughput UniCel® DxI 800 Access Immunoassay System.

P941 Evaluation of the new ARCHITECT CMV IgM, IgG and IgG avidity assays

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Objectives: A panel of new CMV assays for the ARCHITECT instrument has been developed including a CMV avidity assay based on a new technology. The purpose of this study was to compare the performance of the fully automated CMV IgM, IgG and IgG avidity tests on ARCHITECT with other available assays.
Methods: 503 consecutive fresh patient sera (routine sera) received at the University Hospital laboratory for CMV IgM and IgG testing on AxSYM (Abbott), 100 preselected AxSYM IgM positive sera and 96 sera from 33 pregnant women with a recent CMV primo infection (seroconversion sera) were tested for CMV IgM and IgG on the ARHCITECT (Abbott), VIDAS (BioMerieux) and Dade Behring assays. The seroconversion sera

were also tested with the IgG avidity tests developed on ARCHITECT and VIDAS. The specificity of the avidity tests was assessed on 100 preselected AxSYM IgM negative, IgG positive sera.

Results: The relative agreement for the CMV IgM determination on the routine sera between ARCHITECT and VIDAS, Dade Behring and AxSYM was respectively 97%, 94% and 93% for the CMV IgM tests and 99%, 98% and 98% for the CMV IgG tests. CMV IgM reactivity on ARCHITECT but not with the other assays, occurred only in 1 of the 503 routine sera (0.2%). In 3.0% of routine sera CMV IgM was positive only on AxSYM. CMV IgG avidity was high in 50% of routine sera with ARCHITECT CMV IgM reactivity. All preselected AxSYM IgM positive sera with a low VIDAS IgG avidity were reactive on all IgM assays tested. The specificity of the CMV IgG avidity test was 98% for ARCHITECT and 76% for VIDAS. No high CMV IgG avidity test results were found within the first 3 months after seroconversion with both assays. High avidity was attained earlier on ARCHITECT than on VIDAS. In several patients no gradual increase in avidity was observed after seroconversion. This phenomenon was more frequent with the ARCHITECT avidity test compared to the VIDAS assay. The increase in the avidity values after seroconversion needs further validation.

Conclusion: The correlation between the newly developed CMV IgM and IgG tests on ARCHITECT with the VIDAS and Dade Behring assays was excellent (⩾94%) and was highest between ARCHITECT and VIDAS. The CMV IgG avidity test reliably excluded recent infections and showed an excellent specificity (98%).

P942 Earliest possible diagnosis of seroconversion for toxoplasmosis in pregnancy

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The early and definitive detection of a recently acquired infection is critical for the clinical management of the mother and her foetus. IgG antibodies are often absent in early phases of infections and IgM antibodies may be non specific and disappear at the end of pregnancy. Therapy usually given as early as possible could affect antibody production. To distinguish early infection from non-specific antibody response we evaluated two routine tests for IgG and for IGM two new confirmation immunoblots and mmunological tests in 39 pregnant women with suspected seroconversion.39 pregnant women negative for anti-*Toxoplasma* IgG (LIAISON Toxo IgG II DIASORIN Italy, VIDAS toxo IgG II Biomerieux France)and positive for IgM with at least one of the two tests routinely used(LIAISON TOXO IgM DIASORIN Toxo IgM ISAGA BIOMERIEUX)were followed weekly to detect the production of anti-*Toxoplasma* IgG. Spiramicyn was given at the first positive IgM test. All samples were also tested with LDBIO TOXOII IgG and LDBIO TOXOII IgM the latter being not commercially available (LDBIO DIAGNOSTICS France). In 12 out of 39 women lymphocyte stimulation was performed using *Toxoplasma* antigen (DIASORIN)and CD25 and Stimulation Index were evaluated.10 women resulted IgM ISAGA and type II WB negative in all these cases no seroconversion was recorded even after therapy interruption. IgM ISAGA and LDBIO TOXO II IgM was positive in 29 women. For 13 patients the seroconversion was proved with the appearance of specific anti-*Toxoplasma* IgG during the follow-up. In 4 they were already present in the first sample by LDBIO TOXOII IgG in 9 IgG antibodies were detected on subsequent samples earlier with LDBIO TOXOII IgG and with LIAISON Toxo IgG assay.9 were also positive in lymphocyte stimulation.13 women were LDBIO TOXOII IgM negative and no IgG was detected with all the tests on later samples even after the treatment was discontinued. In 5 cases lymphocyte stimulation confirmed these results.

Conclusions: In 36 patients an early and correct diagnosis was reached with LDBIO TOXO II IgM. LDBIO TOXO II IgG and LIAISON Toxo IgG II were positive earlier and confirmed the seroconversion several weeks before the other IgG test. In all negative cases it was possible to stop safely the therapy and to reassure the women. No infected woman was missed, all positive women were given the appropriate therapy and prenatal diagnosis was offered. We had three false positive results at IgM (one also with lymphocyte stimulation).

P943 Performance of five commercial immunoblots for serological confirmation of syphilis

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Objectives: The incidence of syphilis has increased yearly since 2000. Syphilis is famous for its polymorphic presentation and diagnosis is usually established by serology. Nontreponemal test and a specific treponemal test are classically used. Immunoblots (IB) (IgG and IgM) have recently been proposed as confirmatory tests, but without clear indications. Except for Inno-Lia (Innogenetics), performance data are rare. The multiparametric approach is based on antibody responses to 3 immunodominant *Treponema pallidum* recombinant polypeptides (TpN47, TpN17, TpN15) and one synthetic peptide (TmpA) blotted onto strips.

Methods: The present study compared 5 commercial IB IgG tests (InnoLia, Innogenetics; Euroline, Euroimmun; EcoLine, Virotech; Recomblot, Mikrogen; and Virastripe, Servibio) and 3 IgM IBs (InnoLia, Euroline, and Recomblot) in routine. All sera were tested by the rapid reagin plasma test (RPR), the *T. pallidum* hemagglutination assay (TPHA), the immunoglobulin IgG-fluorescent *T. pallidum* absorption assay (IgG-FTA-ABS), the immunoglobulin IgM-fluorescent *T. pallidum* assay (IgM-FTA), and an enzyme immunoassay (IgM-EIA). We tested 44 well-defined sera at different stages of syphilis (clinical and serology findings), 20 sera with an indeterminate serological status for syphilis and 7 samples from subjects with potentially interfering diseases. For 5 patients, we also compared serology results at diagnosis and at 3 to 6 months' follow-up, to investigate antibody kinetics.

Results: All IgG IB assays provided comparable results and confirmed the 44 syphilis. No sera from patients with autoimmune disease or lyme borreliosis were positive. TpN47 and TmpA was the most frequent antigen in all stages of syphilis and band intensity varied with TPHA titer. Anti-TmpA antibody levels tended to fall after treatment, and TNp47 indicated serological scarring.

Whenever ELISA-IgM and FTA-IgM were concordant, the IB IgM test confirmed diagnosis, and identified 4 out of 6 discordant cases (ELISA IgM negative, FTA IgM positive). The recomblot IgM and the InnoLia IgM achieved the highest sensitivities.

Conclusion: The main interest of IB tests lies confirming or ruling out a diagnosis of syphilis in case of discordant screening findings. Further studies are needed to assess application to latent and neonatal syphilis.

P944 Use of the recombinant k39 immunochromatographic strip-test for serological diagnosis of visceral leishmaniasis: experience from Apulia, southern Italy

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Objective: Visceral leishmaniasis (VL) is endemic in our area, Apulia, Southern Italy. The aim of this study was to evaluate a commercially available membrane-based immunoassay (MBI) using a recombinant protein rk39 as antigen in comparison to the immunofluorescent antibody assay (IFA) for the detection of specific anti-*Leishmania* antibodies.

Methods: A total of 264 individuals including 19 patients with VL (3 of whom HIV-positive), 67 with suspected VL and 40 healthy controls were tested. VL diagnosis was made by microscopic demonstration of *Leishmania* amastigotes in Giemsa stained smears from bone marrow aspirates and/or on the presence of specific antibodies. In a HIV-positive patient diagnosis was performed by the PCR on peripheral blood. IFA and MBI were performed in all 264 sera according to manufacturer's instructions.

Results: The MBI was positive in all the 19 patients with VL (diagnosed by the presence of antibodies by IFA and/or the presence of amastigotes in bone marrow aspirate) and negative in sera from the

remaining individuals. The sensitivity and specificity of MBI was 100%, respectively. The IFA failed to detect anti-*Leishmania* antibodies in the first serum sample of a HIV-positive patient but antibodies were detected in a second serum sample obtained 7 days later. On this patient the MBI was positive on the first serum sample tested. The MBI works well also on HIV–*Leishmania* co-infected patients in whom low yield of serological studies may be a most characteristic finding. Follow up performed on 7 patients with VL showed that both antibodies to *Leishmania* detected by IFA and MBI remain at detectable levels up to 12–24 months. A dilution trial performed on sera of 2 patients showed that a positive reaction by MBI was detectable at serum dilution up to 20,480, indicating that a strong immuno-response is mounted against the rk39 antigen.

Conclusions: The MBI is a reliable test for diagnosis of leishmaniasis and is not only a good alternative in a setting where a reliable enzyme immunoassay or IFA tests are not available, but also in well equipped laboratories because positive results can be obtained in 10 minutes. The cost of MBI in our experience was approximately 8.43 euro per test, compared with approximately 12 euro for IFA. MBI appears highly sensitive, specific, rapid and cost-effective for the serological diagnosis of VL in our location where VL has a low endemicity.

P945 Virus-specific antibody activity of different subclasses of immunoglobulins G in Cytomegalovirus infections

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Objectives: Patients with primary, reactivated or convalescent phase of Cytomegalovirus (CMV) infections were examined for antiviral antibodies of the IgG subclasses. The purpose of this study was to investigate the diagnostic value of anti- CMV IgG subclasses for diagnosis of acute CMV infection.

Methods: A total of 52 serum samples from patients with different phases of CMV infection were tested for anti-CMV IgG, IgM and IgG avidity by commercially available enzyme immunoassay (RPC “Diagnostic systems”, Russia). Anti CMV IgG specific to envelope glycoprotein (gB) were studied to confirm recent infections. The reactivity pattern of IgG subclasses to individual CMV proteins (pp150, pp52, pp38, pp28) were analysed with subclass specific monoclonal antibodies from Zymed Laboratories Inc., USA.

Results: The distribution of CMV-reactive IgG subclasses among groups with different CMV infection phases was analysed. CMV-specific IgG1 was predominant in all groups. In primary infection frequency of anti-CMV IgG3 is significant higher (75%) than in latent phase (13%, P = 0.001) and reactivated CMV infection (23%, P = 0.032). Frequency of anti-CMV IgG4 is significant higher (62%) for reactivated CMV infection than for convalescent phase (10%, P = 0.001). No difference was observed in total IgG subclass value in all analysed groups.

No significant difference was found for IgG2 and IgG4 reactivity to individual CMV proteins between studied groups. In primary infection anti-pp28 IgG1 was detected rarer (25%) than in reactivated CMV infection (85%, P = 0.018). Among patients with acute CMV infection (recent or reactivated) frequency of anti-pp150 IgG3 positive sera is significant higher (38%) than in group of latent infection (0%, P = 0.001, P = 0.003).

Conclusions: Frequencies of anti-CMV IgG4 and anti-pp150 IgG3 are different for acute and latent CMV infection and anti-CMV IgG3 and anti-pp28 IgG1 – for primary and reactivated CMV infection. The measurement of IgG subclasses reactive with individual CMV proteins may be a sensitive indicator of CMV infection phases.

P946 Significance of laboratory findings for the diagnosis of neurosyphilis

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Objectives: The usage of recombinant *T. pallidum* antigens in ELISA and passive hemagglutination assay (TPHA) has the potential for improvement lab diagnostics of neurosyphilis. The aim of this study was to evaluate the diagnostic relevance of cerebrospinal fluid (CSF)-tests, to analyse antibody reactivity pattern in CSF from neurosyphilis patients by ELISA and TPHA and new criterion of the intrathecal production of specific IgG definition.

Methods: Four full-length recombinant proteins (TmpA, 47, 17, 15 kDa) were expressed in *E. coli* and then used individually to develop ELISA and TPHA tests. The FTA-ABS, TPI, ELISA, TPHA, VDRL, CFT (complement fixation test) tests were performed on CSF samples from 22 patients with a diagnosis of active neurosyphilis, from 4 patients treated for neurosyphilis and from 17 patients without syphilitic CNS involvement. TPHA-IgG titres have been determined in paired serum and CSF samples from 13 neurosyphilis patients and 1 patient without syphilitic CNS involvement.

Results: When CSF was used as diagnostic fluid sensitivities of FTA-ABS, ELISA, TPHA (82.3%, 86.4%, 86.4% accordingly) were higher in comparison with TPI, VDRL, CFT tests (62.5%, 58.8%, 60% accordingly). However, VDRL and CFT demonstrate 100% specificity in contrast with treponemal tests (specificity 75–84.6%). The best result matched with clinical diagnosis of neurosyphilis was demonstrated by next three combinations: FTA-ABS+ELISA+TPHA, FTA-ABS+ELISA+VDRL or FTA-ABS+TPHA+VDRL – 88.4%.

Antibodies specific to 17 kDa (88.9–90.9%) and TmpA (72.2–86.4%) were prevalent in CFS samples. There is no correlation in titers of antibodies specific to individual *T. pallidum* recombinant antigens in paired serum and CFS samples from patients with neurosyphilis. In the same time strong correlation was observed when paired CFS/serum samples from patient without syphilitic CNS were tested.

Conclusions: Different antibody reactivity pattern in paired CFS/serum samples may indicate intrathecal production of antitreponemal antibodies.

P947 Sensitivity and mutant recognition of the DiaSorin liaison HBsAg assay

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Objectives: Determination of HBsAg is still indispensable for the diagnosis of HBV infection despite additional anti-HBc screening and NAT. Yet, high sensitivity and mutant recognition are the prerequisites for HBsAg detection. The European regulations require an overall sensitivity equivalent to an established device, early detection to be state of the art and recognition of virus variants. The Liaison HBsAg assay was challenged for analytical sensitivity, seroconversion sensitivity and frequently occurring HBV mutants.

Methods: Diagnostic and analytical sensitivity of the Liaison HBsAg assay were assessed using 37 commercial seroconversion panels, internationally recognised HBsAg standards (PEI HBsAg ad and ay, 2nd WHO HBsAg standard and HBsAg mutant samples (4 recombinant constructs S143L (ayw), C76Y/R169H, S53L/ I126N/ S210N, C76Y/ T143M/ F219S and 4 naturally occurring mutants G129H, M133T, G145R, T143L).

Results: Seroconversion data revealed that the Liaison HBsAg assay showed an overall clinical sensitivity in the range of European CE-marked HBsAg tests. Among the 20 HBsAg tests assessed by PEI it ranked at position 9. Analytical sensitivities of the Liaison HBsAg were 0.023 PEI U/ml relating to the PEI HBsAg ad standard and 0.038 PEI U/ml for the HBsAg ay standard. Based on the 2nd WHO HBsAg standard analytical sensitivity was 0.049 IU/ml. In addition, the Liaison HBsAg test detected all natural mutants as well as all recombinant constructs investigated.

Conclusions: Sensitivity of Liaison HBsAg assay meets the current state of the art in the sense of the European regulations. Analytical sensitivity of the Liaison HBsAg complies with the revised European requirement for 0.130 IU/ml relating to the 2nd WHO HBsAg standard. Seroconversion sensitivity is in a good range with other CE-marked assays. The assay shows no deficiencies in the detection of frequently occurring HBsAg mutants.

P948 **Bacteriological and serological aspects of *Chlamydophila pneumoniae* pharyngotonsillitis in adults**

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Objective: Acute pharyngotonsillitis is one of the most common infections encountered by family physicians. Most patients with acute pharyngotonsillitis have symptoms that can be attributed to infection with a respiratory virus, such as adenovirus, influenza virus, parainfluenza virus, rhinovirus, and respiratory syncytial virus. However, in approximately 30% to 40% of cases, acute pharyngotonsillitis is of bacterial aetiology. Group A beta-hemolytic streptococci (GABHS) are responsible for most bacterial cases of acute pharyngotonsillitis, although other pathogens, such as *Neisseria gonorrhoeae*, *Arcanobacterium haemolyticum*, *Mycoplasma pneumoniae*, and *Chlamydophila pneumoniae*, may be the causative agents in sporadic cases.
To assess whether *C. pneumoniae* plays a role in pharyngotonsillitis, the prevalence of *C. pneumoniae* detection in adult patients with upper respiratory illnesses was investigated.
Methods: Clinical samples from 98 adult patients were sent to our laboratory from family physicians between January 2006 and March 2007. All clinical and serum samples were collected from patients who were diagnosed with pharyngotonsillitis based on clinical symptoms. During the same period, we enrolled 75 healthy subjects without any history of respiratory tract infections in the 3 months before enrolment. The nasopharyngeal swabs were evaluated for isolation of *C. pneumoniae* in cell culture, all specimens were passaged three-times. The presence of chlamydial inclusions was examined by Immunofluorescent (IFA) staining and Giemsa staining.
A commercial microimmunofluorescence (MIF) test was used to measure *C. pneumoniae*-specific IgG, IgM, and IgA antibodies (Labsystems, Helsinki, Finland distributed by Dasit, Italy).
Results: *C. pneumoniae* was detected by isolation in ten patients (10.2 per cent) but in none of the control. Immunofluorescent (IFA) staining and Giemsa staining of 98 pairs of matched swabs detected *C. pneumoniae* in three by both methods, in four by Giemsa staining alone, and in three by IFA alone.
Twenty-four (24.5 per cent) of 98 patients had a positive result for *C. pneumoniae* IgG antibodies and nine (9.2 per cent) for *C. pneumoniae* IgA antibodies. No patient had a *C. pneumoniae* IgM antibodies.
Conclusion: Our results suggest that the therapeutical choice of acute pharyngotonsillitis in adults should take into account the possibility of *C. pneumoniae* infections.

P949 **Laboratory evaluation of UniCel DxI 800 analyser (Beckman Coulter) for HBV and HCV serological markers**

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Objectives: The UniCel DxI 800 analyser (Beckman Coulter) is a multiparametric immunoassay system with random access that uses magnetic particules separation and indirect chemiluminescent technology.
In this study, intra-assay and inter-assay precision, and a comparison with the Vitros ECi (Ortho Clinical diagnosis) were evaluated for the following virological markers: antibody to hepatitis C virus (anti-HCV) and four hepatitis B virus (HBV) markers i.e. HBsAg, anti-HBc, anti-HBc IgM and anti-HBs.
Methods: Intra-assay precision was determinated by measuring within one run 20 replicates of the manufacturer positive controls. For inter-assay precision, the same controls were tested daily during the evaluation period in 26 to 36 runs depending on the markers.
Comparison of the two analysers was assessed by testing patients sera previously analysed in our laboratory with the Vitros ECi and stored at $-23\pm5^{\circ}$C. Negative and positive samples were compared: respectively 104 and 108 for HBsAg, 120 and 133 for anti-HBs, 225 and 36 for anti-HBc, 103 and 21 for anti-HBc IgM and 109 and 26 for anti-HCV
For the quantitative anti-HBs assay, correlation with the Vitros was determined with the Spearman regression test. Moreover linearity, accuracy and detection limit were evaluated using serial dilutions (from 500 mUI/ml to 10 mUI/ml) of the WHO internationnal standard for anti-HBs (W1042).
Results: Coefficient of variation for intra-assay and inter-assay tests were respectively 0.8% and 4.4% for HBsAg, 2.1% and 5.1% for anti-HBs, 2.4% and 6.2% for anti-HBc, 5% and 8.7% for anti-HBc IgM and 3.7% and 7.4% for anti-HCV.
The concordance rates between the two methods were high: 100% for the five markers except for negative HBsAg sera where one discordant result was observed (99% concordance). Anti-Hbs results appeared statistically correlated either with Vitros results or with the WHO standard ($p<0.01$ with Spearman test). All the five replicates of the 10 mUI/ml sample were detected. The throughput of the analyser was estimated at about 200 tests per hour.
Conclusion: The results of this evaluation confirmed that the five assays meet the criteria of the Common Technical Specifications of the European Union's Directive on In Vitro Diagnostic Medical Devices in terms of specificty and sensitivity. Together with a high throughput, the DXI provides a useful tool for serological diagnosis of HCV anc HBV infection in routine practice.

Host microbial interactions

P950 **Tifacogin and a synthetic TFPI peptide fragment suppress cytokine production due to *Streptococcus pneumoniae* and *Staphylococcus aureus* in whole blood cultures**

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Objective: Tifacogin, rTFPI, has demonstrated efficacy in acute inflammation after *Escherichia coli* infection in several animal models of severe sepsis. Tifacogin is currently being evaluated in a phase 3 clinical trial in severe community acquired pneumonia patients. Our goal was to investigate tifacogin activity in bacterially-induced inflammatory disease in an ex vivo model using disease relevant bacteria. The objective of this study was to investigate if cytokine production was reduced by tifacogin and TFPI-derived peptides in an ex vivo model of blood cultures inoculated with *S. pneumoniae* or *S. aureus*.
Methods: Tifacogin and synthetic C-terminal peptides derived from TFPI were prepared and their effect on inflammatory cytokine production in 10% human blood cultures containing Gram+ pathogens including *S. pneumoniae* and *S. aureus* were measured with and without vancomycin. For cytokine analysis, whole blood assay reactions were incubated for 18–20 hours and cell-free supernatants were harvested. Aliquots were used in a custom pro-inflammatory human cytokine panel. Bacterial titers were measured by serial dilution of cultures, plating on appropriate agar and incubation overnight at 37°C.
Results: Addition of tifacogin to vancomycin reduced inflammatory cytokines IL-1B (7 pg/mL compared to 36 pg/mL for vancomycin alone), IL-6 (1100 pg/ml compared to 3450 pg/mL), TNF-a (37 pg/ml compared to 250 pg/mL), and IL-8 (3000 pg/mL compared to 25000 pg/mL) respectively compared to vancomycin alone. Similar changes were noted with other anticoagulants suggesting the effect was tied to clotting inhibition. In combination with vancomycin, a TFPI peptide but not its scrambled control also significantly reduced IL-6, IL-8 and TNF-a levels compared to vancomycin alone. The effects of tifacogin and TFPI peptide reinforced each other to lower cytokines to near baseline levels, suggesting different underlying mechanisms. Similar findings were noted when erythromycin was substituted for vancomycin.
Conclusions: Our results show that tifacogin and an rTFPI peptide demonstrate anti-inflammatory effects by reducing cytokine levels in blood inoculated with *S. pneumoniae* or *S. aureus*. These data suggest that tifacogin has antiinflammatory activity in addition to previously demonstrated anticoagulant activity. Tifacogin and rTFPI peptide fragment may have adjunctive antiinflammatory activity in combination with antibiotics.

P951 IL-17 is associated with severity of invasive meningococcal disease

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Objectives: The aim of the present study was to determine the cytokine profile of plasma and cerebrospinal fluid (CSF) during invasive meningococcal disease (IMD) with use of a multiplex system. The relationship between cytokine concentrations and IMD severity was also assessed.

Methods: A total of 12 patients with IMD were evaluated. Paired plasma and CSF samples were obtained at the time of diagnostic (day 1) and follow-up (day 3–5) lumbar puncture (LP). Plasma and CSF concentrations of 11 biomarkers (TNF-α, IL-1β, IL-6, IL-8, IL-10, IL-12, IL-17, MCP-1, MIP-1β, IL-1Ra, leptin) were analysed using a Luminex® analysis. Disease severity was evaluated using Acute Physiology and Chronic Health Evaluation (APACHE) II score, Sequential Organ Failure Assesment (SOFA) score and Glasgow Coma Scale (GCS) score.

Results: Plasma concentrations of IL-6, IL-10, IL-17 and IL-1Ra were associated with APACHE II on day 1. With regard to the severity of organ dysfunction on day 1, plasma IL-6, IL-8 and IL-10 concentrations showed the best correlation with SOFA. Unlike plasma cytokines, CSF concentrations were not associated with the disease severity.

Conclusion: Multiple cytokine analysis revealed cytokine patterns associated with inflammatory response during IMD. Moreover, the study is first to demonstrate that the increased IL-17 plasma concentration is associated with IMD severity. Since IL-17 is mainly produced by a CD4+ T cell subset (Th17 cells), our results suggest an important role of these cells in the IMD pathophysiology. Acknowledgement. The study is supported by a grant IGA NR/9316–3.

P952 Waddlia chondrophila multiplies within human macrophages in a vacuole associated to the endoplasmic reticulum

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Objectives: Waddlia chondrophila is an obligate intracellular bacteria which belongs to the Chlamydiales order. It was isolated twice from aborted bovine foetuses, first in 1990 in the United States and then in 2002 in Germany. A serological study supported the abortigenic role of Waddlia in bovine species and inoculation of a pregnant cow with this intracellular bacteria was associated with foetal death within 2 weeks. Recently, we observed a strong association between the presence of anti-Waddlia antibodies and human miscarriage. To further investigate the pathogenic potential of W. chondrophila in humans, we studied the entry and the multiplication of this obligate intracellular bacteria in human macrophages.

Methods: Monocyte-derived macrophages were incubated with living or heat-inactivated bacteria for 15 min at 37°C after a centrifugation step. Cells were then washed and further incubated for different periods at 37°C. Entry was assessed by confocal microscopy and bacterial growth was quantified using immunofluorescence with mice anti-Waddlia antibodies and real-time PCR. Intracellular traffic of W. chondrophila in human macrophages was assessed by studying the co-localisation of the bacteria with various cell markers.

Results: Confocal microscopy confirmed that W. chondrophila is able to enter into human monocyte-derived macrophages. W. chondrophila organisms were shown to multiply readily within macrophages. The proportion of infected macrophages increased from 13% at day 0 to 96% at day 4, and the mean number of bacteria per macrophage (assessed by immunofluorescence) increased of more than 2 log in 4 days. This growth was confirmed by real-time PCR with a 3 log increase in 4 days and was associated with a decrease in the number of macrophages. As expected, the heat-inactivated bacteria, which also entered into the macrophages, did not increase over time and exhibited no cytopathic effect. Using immunofluorescence and confocal microscopy, we showed that W. chondrophila escape the endocytic pathway and co-localise with markers of the endoplasmic reticulum.

Conclusion: This study shows that W. chondrophila enters and multiplies logarithmically within human macrophages suggesting its possible human pathogenicity.

P953 *Streptococcus pneumoniae*, *Neisseria meningitidis* and *Haemophilus influenzae* elicit distinct patterns of inflammatory parameters in cerebrospinal fluid of patients with bacterial meningitis

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Objectives: Morbidity and mortality of meningitis caused by *S. pneumoniae*, *N. meningitidis*, and *H. influenzae*, the three major causes of bacterial meningitis, differ dramatically with *S. pneumoniae* causing the highest incidence of neurological disabilities and death. Pathogenetic mechanisms underlying these differences may involve inflammatory reactions of the brain in response to the different invading pathogens. Inflammatory mediators in cerebrospinal fluid (CSF) from patients suffering from bacterial meningitis caused by the three different bacterial species were assessed with the aim to identify inflammatory mechanisms that contribute to the pathogen related differences in outcome.

Methods: A defined panel of inflammatory parameters were assessed in CSF from patients with confirmed meningitis due to *S. pneumoniae* (n = 32), *N. meningitidis* (n = 38), or *H. influenzae* (n = 3). Concentrations of cytokines, chemokines and matrix-metalloproteinase-9 (MMP-9) were assessed by a microsphere-based multiplex assay. In addition, the concentration of MMP-9 was determined by gelatine gel zymography. The activity of nitric oxide synthase was determined by measuring nitrate/nitrite levels.

Results: IL-2 (Kruskall-Wallis $p<0.03$), IFN-gamma ($p<0.03$), and MCP-1 ($p<0.01$) were found to be differentially up-regulated during bacterial meningitis caused by the three pathogens, with *S. pneumoniae* triggering the highest, and *H. influenzae* the lowest CSF concentrations of these cytokines. CSF levels of MMP-9 also differed among the pathogens (one way analysis of variance $p<0.001$) with levels in pneumococcal meningitis being significantly higher than in meningitis caused by *H. influenzae* (Bonferroni's $p<0.05$). Nitric oxide synthase activity in CSF did not differ for the three causative pathogen.

Conclusion: From the inflammatory parameters assessed, concentrations of IL-2, IFN-gamma, MCP-1, and MMP-9 were significantly higher in CSF samples from patients with meningitis caused by *S. pneumoniae* compared to *H. influenzae* and *N. meningitidis*. The higher case fatality rates and incidence of neurological sequelae caused by pneumococcal meningitis compared to meningococcal or *H. influenzae* meningitis may be associated with an increase in these inflammatory parameters.

P954 The host response in pneumococcal meningitis: differential transcriptome analysis in cerebral cortex and hippocampus

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Background: For bacterial meningitis (BM) treatment options are insufficient as evidenced by a high mortality of up to 30% and the occurrence of neurological sequelae in up to 50% of the surviving patients. Knowledge on the diseases processes is prerequisite for improvements in therapy. By setting the global gene expression profile in the context of biological meta-data resources we aimed to unravel the pathological processes which determine the outcome of bacterial meningitis.

Methods: An infant rat model of experimental pneumococcal meningitis (PM) was used. Brain regions prone to injury e.g. the cortex and the hippocampus were dissected form animals in acute and late phase of the disease e.g. at 24 and 72 h after infection. Gene expression profiles were assessed with Affymetrix GeneChip® Rat 230 2.0 arrays. Significance of transcriptomic changes were calculated with a moderated Bayesian t-statistics using the limma package. GO statistics was performed by the GOstats software packages provided by the Bioconductor project.

Results: From 31,000 transcripts of the rat genome represented on the chip, approximately 17,000 were detected in both control and infected

animals. 3600 genes were found to be significantly (FDR Benjamini-Hochberg $p \leqslant 0.05$) regulated at 24 h.p.i and approx. 1100 genes at 72 h.p.i.
Gene Ontology statistics revealed that genes which were regulated at both time points after infection were mainly involved in immunological and inflammatory processes. This observation applies for both the cortex and the hippocampus. In the cortex the dominant process was angiogenesis at 24 h, while it was neurotransmitter signalling at 72 h after infection. Genes found to be regulated in the hippocampus at 24 h.p.i were mainly involved in apoptosis shifting to neurogenesis at 72 h.p.i.
Conclusion: By integrating differential gene expression data in the context of biological research databases this study expands the current knowledge of pathways of injury and regeneration in BM. This approach identified angiogenesis, neurotransmitter signalling, apoptosis and neurogenesis as targets for the development of new therapeutic strategies.

P955 Apoptosis of T lymphocytes in the culture of peripheral blood mononuclear cells from patients with late Lyme arthritis

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Objectives: Apoptosis of activated T lymphocytes is essential in physiologic regulation of inflammatory and immune response. Lymphocyte susceptibility to apoptosis may be increased in immune deficits and decreased in autoimmune disorders. The pathogenesis of chronic articular symptoms in Lyme borreliosis (Lyme arthritis – LA) is unclear and has been hypothesised to involve aberrant immune response, including possible autoimmunity. We studied apoptosis of peripheral T lymphocytes obtained from patients with late LA and cultured in presence of *Borrelia burgdorferi* sensu lato (*B. burgdorferi*) antigens. We checked for abnormalities in apoptosis rate, especially for decreased susceptibility to apoptosis which might be a hallmark of uncontrolled inflammatory/immune response or autoimmunity.
Methods: Peripheral blood mononuclear cells (PBMC) from 23 subjects: 14 patients with LA persistent or recurrent for > 6 months (LA group) and 9 persons with no clinical suspicion or after exclusion of Lyme borreliosis (control group – C) were incubated for 48 hours with no stimulation or with addition of non-viable *B. burgdorferi* spirochetes: *Borrelia afzelii* VS 46110 (B.a.), *B. garinii* 20047 (B.g.) or *B. burgdorferi* sensu stricte B-31 (B.ss.) as antigenic stimulation. Fraction of T lymphocytes (CD3+ cells) undergoing apoptosis was measured by flow cytometry with annexin-V binding assay.

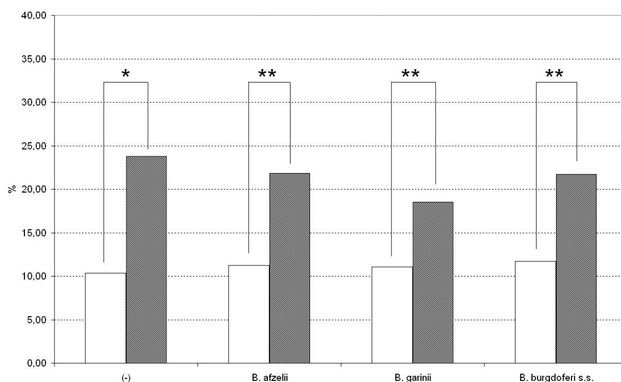

Figure. Fraction of apoptotic T cells (median) in the 48-hour culture either unstimulated (–) or stimulated with antigens of three *B. burgdorferi* genospecies. Empty bars – healthy controls, filled bars – Lyme arthritis patients: $^*p < 0.05$, $^{**}p < 0.01$.

Results: There was a significantly higher fraction of annexine-V positive (apoptotic) T cells in LA (median values – 18–25%) than in C group (10–12%), both with and without antigenic stimulation (Fig). The T cell apoptosis tended to be increased in presence of *B. burgdorferi*, but the effect was weak and statistically significant only in the study population analysed as a whole (LA + C), with rate of apoptosis rising under antigenic stimulation by a median of 10–17% of the baseline (i.e of the value found in unstimulated culture).
Conclusions: No proof of decreased apoptosis of T lymphocytes in late LA was found. Peripheral CD3+ cells from LA patients appear to be more prone to apoptosis in culture, independently of the exposition to *B. burgdorferi*. The role of this phenomenon in the pathogenesis of LA is not clear and may require further study. Some pro-apoptotic effect of *B. burgdorferi* s.l. on human CD3+ cells was observed, which may reflect spirochete's natural defence mechanism against host's immune system.

P956 Nramp1 limits the intracellular growth of *Salmonella typhimurium* by impairing bacterial iron uptake and strengthening macrophage effector functions

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Objectives: Natural-resistance associated macrophage protein 1 (Nramp1) is a phagolysosomal transmembrane protein which acts as a transporter for protons and divalent ions including iron. Nramp1 functionality is associated with an increased resistance against various intracellular pathogens including leishmania, mycobacteria and salmonellae.
Methods: We investigated the regulation of iron homeostasis and immune function in RAW264.7 macrophages stably transfected with functional or non-functional Nramp1 in response to infection with *Salmonella typhimurium*, the causative agent of murine typhoid fever.
Results: Macrophages bearing functional Nramp1 displayed a reduced expression of transferrin receptor 1, resulting in the decreased acquisition of transferrin-bound iron. In contrast, the expression of the transmembrane iron-exporter, ferroportin 1, and in parallel cellular iron release was significantly higher in *Salmonella*-infected Nramp1-functional macrophages as compared to phagocytes lacking Nramp1. Conceivably, Nramp1 functionality caused a reduction in the cytoplasmatic iron pool within macrophages. Additionally, Nramp1-expressing macrophages were capable of limiting the intracellular persistence of *S. typhimurium* significantly better as compared to cells non-functional for this protein. This antimicrobial effect of Nramp1 was characterised by the reduced iron access by engulfed *Salmonella* and the increased formation of nitric oxide, tumour necrosis factor and interleukin-6 by infected phagocytes.
Conclusions: Our data suggest that the protective effects of Nramp1 may be partly attributable to its capacity to reduce the iron availability within phagocytes, an effect which limits microbial iron access while concomitantly strengthening macrophage immune effector functions.

P957 Interferon-gamma limits the availability of iron for intramacrophage *Salmonella typhimurium*

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Objectives: In stimulating effector functions of mononuclear phagocytes, interferon-gamma (IFN-γ) is of pivotal importance in host defence against intramacrophage pathogens including salmonellae. As the activity of IFN-g is modulated by iron and since a sufficient availability of iron is essential for the growth and proliferation of pathogens, we investigated the regulatory effects of IFN-γ on iron homeostasis and immune function in murine macrophages infected with *Salmonella enterica* serovar Typhimurium (*S. typhimurium*).
Methods: We investigated the effects of IFN-γ on iron homeostasis and immune function in RAW264.7 macrophages in response to infection with *Salmonella typhimurium*.
Results: In *S. typhimurium*-infected phagocytes, IFN-γ caused a significant reduction of iron uptake via transferrin receptor 1. In parallel,

we observed an enhanced efflux of iron from *Salmonella*-infected macrophages upon activation with IFN-γ, which could be traced back to an enhanced expression of the iron exporter ferroportin 1. Moreover, the expression of haem oxygenase 1 and of the siderophore-capturing antimicrobial peptide lipocalin 2 was markedly elevated following bacterial invasion, with IFN-γ exerting a super-inducing effect. This observed regulatory impact of IFN-γ reduced the intracellular iron pools within infected phagocytes, thus restricting the acquisition of iron by engulfed *S. typhimurium* while concomitantly promoting NO and TNF-α production.
Conclusion: Our data suggest that the modulation of crucial pathways of macrophage iron metabolism in response to IFN-γ concordantly aims at withdrawing iron from intracellular *Salmonella* and at strengthening macrophage immune response functions. We therefore propose that these regulations reported herein reflect novel antibacterial effects of IFN-γ being consistent with the principles of nutritional immunity.

P958 Inflammatory cell infiltration and inflammatory cytokines: indicators of *Streptococcus agalactiae* infection in experimental mouse mastitis

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Streptococcus agalactiae is a major contagious pathogen causing mastitis highly adapted to survive in bovine mammary gland.
Objectives: This study focuses on the use of a mouse model of *Streptococcus agalactiae*-induced mastitis as a practical approach for regarding the pathogenesis of the bacteria.
Methods: BALB/c mice in 10–15 th day of lactation were intramammary (i.ma) challenge on both L4 (on the left) and R4 (on the right) abdominal mammary glands with 10^8 cells of *S. agalactiae* isolated from bovine mastitis (infected animals) or with PBS (control animals). Throughout the study the colonisation was evaluated by bacterial counts (CFU) in the mammary gland, kidneys, spleen and liver. Mammary tissue alterations were evaluated by haematoxylin-eosin staining of mammary sections. Cytokine production in the mammary gland during infection was evaluated by ELISA.
Results: *S. agalactiae* i.ma infection showed that the bacteria replicates in the mammary gland and peaked 24 h later. At the same time, a massive infiltration of polymorphonuclear cells (PMNs) and an increase in IL-1β, IL-6 and TNF-α (inflammatory cytokines) levels were detected in the mammary gland. After this, a gradual decrease on bacteria load was observed which was accompanied by a decrease in the number of PMNs and an increase of macrophages and lymphocytes, indicating an evolution into a chronic process in the mammary tissue. A decrease in the levels of TNF-α, IL-1beta and IL-6 were observed in the mammary gland 72 h after infection, which was accompanied by an increase in the levels of IL-12 and IL-10. Dissemination of the bacteria from the mammary glands to the kidneys, spleen and liver was observed 6 hours after infection, with a peak of the CFU after 12 hours for the kidneys and spleen and after 24 hours for the liver. The bacterial load in these organs was significantly lower than the one observed in the mammary gland, throughout the study.
Conclusion: The mouse model of infectious mastitis proposed here is suitable and less costly than cows for the study of pathogenesis of *S. agalactiae* and it can be used as a tool for evaluating potencial vaccines against mastitis induced by *S. agalactiae*.
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P959 Crucial role of IFN-gamma receptor in murine *Legionella longbeachae* infection

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Objectives: *Legionella longbeachae* serogroup 1, a facultative intracellular pathogen is a causative agent of life-threatening pneumonia. Human infection is particulary common in Australia, but sporadic infections have been documented in other countries. Previous studies have shown that activation of U937 macrophages with interferon-gamma (IFN-g) restricts the growth of *L. longbeachae*. In this study we decided to explore the role of IFN-g receptor in *L. longbeachae* mice infection. We explored the kinetics of bacterial multiplication and patohistological changes in IFN-gR –/– knock out and C57Bl/6 mice.
Methods: Pathogen-free 6- to 10-weeks-old IFN-gR –/– and C57Bl/6 (control) mice were infected by intratracheal inoculation with *L. longbeachae* serogroup 1 (clinical isolate D4968) using a dose of 10^3 CFU. We determined the mortality rate and bacterial clearence from the lungs of infected mice at different time points post infection. We also followed the patohistological changes in these organs during 72 hours of infection.
Results: Our results showed a dramatic difference in the mortality rate between IFN-gR –/– and control C57Bl/6 mice. After intratracheal infection with 10^3 CFU of *L. longbeachae* all IFN-gR –/– mice died within 5 days, while control mice survived the infection. Mice infected with 10^3 CFU of *L. longbeachae* were not able to overcome infection for more than 5 days. In contrast, control mice could reduce the bacterial burden in the lungs below the assay detection level 14 days p.i. They, however, showed focal peribronchiolar inflammatory cell infiltration with basement membrane injury, three to seven days after infection. On the other hand, IFN-gR –/– mice developed severe bronchopneumonia with destruction of the alveolar wall and infiltration of the interstitial tissue within neutrophils and lymphocytes three days after infection.
Conclusion: Our results shows that IFN-g receptor plays a crucial role in the host defence against *L. longbeachae* serogroup 1 infection. The findings suggest that retardation of the host immune response caused by deficiency of IFN-gR, might allow bacteria to grow and cause fulminant pneumonia.

P960 The degree of invasiveness of *Campylobacter jejuni* influences cytokines induction in the human monocytic cell line THP-1

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Background: *Campylobacter jejuni* can cause enteritis that is associated with an acute inflammatory response. *C. jejuni* can penetrate the intestinal epithelial barrier and interact with leucocytes, inducing proinflammatory cytokines. The severity of clinical symptoms is related to strain differences. We studied the in vitro invasiveness of eight human isolates of *C. jejuni*, which possess three, two, or none of the virulence genes cdtB, ciaB and iam, with the human monocytic cell line THP-1 and determined the levels of cytokines (IL-1, IL-6, IL-8, IL10, IL 12 and TNF) induced.
Methods: The invasion assay was carried out in 24 wells plate with a multiplicity of infection of 200:1 for 90 minutes followed by treatment with Gentamicin for 120 minutes. Cells were lysed with 0.01% triton and plated to determine the intracellular organisms /cell. Semi quantitative RT-Multiplex PCR was used to assess the kinetics of cytokine mRNA expression in similarly infected cells and ELISA for cytokine levels.
Results: Invasiveness directly correlated with possession of virulence genes, with the most invasive isolates possessing all three genes, followed by those possessing cdtB and ciaB, followed by those with cdtB and iam, while the least invasive were those lacking all three genes. As for cytokine mRNA expression, TNF was most significantly correlated with invasiveness, with IL-1 and IL-8 closely following, while expression of IL-6, IL-10 and IL-12 was essentially similar for all isolates. Cytokine protein expression on the other hand showed a significant difference between one of the most invasive and least invasive of the isolates with respect to TNF and IL-8 and lower differences with respect to IL-1.
Conclusion: *C. jejuni* infection of monocytoid cells triggers expression of proinflammatory cytokines, most significant of which is TNF. This appears to correlate with the virulence of the isolate and might be responsible for the severity of pathology and symptoms.

P961 **The Toll-like receptor 2 agonist Pam3CSK4 induces microglia-mediated neuronal cell death in vitro**

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Objectives: Microglial cells, the major constituents of innate immunity within the brain, express Toll-like receptors (TLR) recognising exogenous and endogenous ligands. Upon treatment with agonists of TLR 2, TLR 4, and TLR 9, primary mouse microglial cells change their morphology and release nitric oxide and inflammatory cytokines. Activated microglia can injure neurons. We investigated the influence of TLR 2-activation on neuronal viability in vitro.
Methods: Primary mouse cortical neurons and co-cultures of primary mouse cortical neurons and primary mouse microglial cells (ratio 2:1) were treated with the TLR 2-agonist Pam3CSK4 (Tripalmytoyl-cysteinyl-seryl-(lysyl)3-lysine; 10 μg/ml) in combination with interferon-gamma (100 U/ml) for 72 hours. Control cultures were exposed to interferon-gamma alone for 72 hours. Cells were fixated in 4% formaldehyde and stained with the neuronal marker Map-2, the microglial marker isolectin B4, and haemalum. Viability of neuronal cells was assessed by a blinded observer and by measurement of the Map-2-stained area using the image processing and analysis program WCIF ImageJ. Student's t-test was perfomed to analyse differences between groups.
Results: Treatment with Pam3CSK4 did not affect the viability of neurons in the absence of microglial cells (Map-2 stained area: 102% in Pam3CSK4-treated cultures versus 100% in control cultures, $p = 0.904$). In co-cultures of neurons and microglial cells, however, treatment with Pam3CSK4 led to visible neuronal damage reflected by a significant reduction of the Map-2 stained area of approximately 20% ($p<0.05$).
Conclusion: The TLR 2-agonist Pam3CSK4 induces microglia-mediated neuronal damage in vitro. During bacterial meningitis, TLR 2 is stimulated by bacterial products, e.g. bacterial lipoprotein. The activation of microglial cells by agonists of TLR 2 might contribute to the neuronal damage in patients with bacterial meningitis leading to an impaired clinical outcome.

P962 **Role of invariant NKT lymphocytes in the hepatic granulomatous response to *Leishmania donovani* infection**

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Objectives: Visceral leishmaniasis (VL) is a life-threatening infection due to protozoan parasites from the *Leishmania* genus. *L. donovani* in India and East Africa, and *L. infantum* in the mediterranean basin as well as in Brazil are the responsible species for VL. During VL, the hepatic granulomatous response is correlated to the parasitic clearance. However, cellular organisation of mature granulomas is still not totally understood, but seems to involve NKT cells. This particular cell population, at the interface of innate and acquired immunity harbours surface molecules from both NK and T (TCRab) cells. Among NKT cells, invariant NKT lymphocytes (iNKT) are hepatic parenchymatous cells that harbours a semi-invariant TCR, Valpha14-Jalpha18, associated to restricted beta chains. Here, we analyse their role in the granulomatous response during liver infection with *L. donovani* in a murine model deleted in iNKT cells.
Methods: C57BL/6 Ja18–/– mice and congenic wild-type mice were infected with *L. donovani* promastigotes. At days 15, 30 and 60, groups of 4 mice were sacrificed in order to analyse:
– the hepatic parasitic load on May Grunwald-Giemsa stained smears,
– the hepatic granulomatous response on hemalun-eosin-safran stained histological sections,
– the hepatic damage that was evaluated by dosing alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactico-dehydrogenase and alcaline phosphatase levels in the serum.
Results: Compared to wild-type mice that rapidly control the infection, iNKT–/– mice presented with significantly higher hepatic parasitic loads until 10-fold more, at D15 and D30. In parallel, the histologic examination of liver sections displayed a delay in the granuloma maturation. iNKT–/– mice presented at D30 with few large granulomas (13.3% with > 25 cells) versus 33.6% in wild-types, and granuloma maturation was reached in 20% of them versus 50%, respectively. At D60, 40% of the granulomas were matures in iNKT–/– mice and the parasitic loads declined until similar results to wild-types. Hepatic enzyme dosages showed a similar hepatocyte damage in both groups without statistically significant difference.
Conclusion: Finally, our data underline the role of iNKT lymphocytes in the early and efficient organisation of hepatic granuloma that warrants a clearance of parasites. In C57/Bl6 mice that exhibit a resistant phenotype to *Leishmania* infection, the settlement of the adaptative response allows a definitive cure of the infection.

P963 **Iron overload favours the elimination of *Leishmania infantum* from mouse tissues by enhancing the production of reactive oxygen species**

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Objectives: We have previously determined that iron overload decreases the growth of *Leishmania infantum* in the liver and spleen of a susceptible mouse strain. The purpose of this work was to investigate if the inhibitory effect of iron overload on the growth of this protozoan is dependent on the production of reactive oxygen species by the macrophagic NADPH oxidase.
Methods: To achieve this goal, we used mice genetically deficient in the p47phox subunit of NADPH oxidase (p47phox knock-out). Control and knock-out mice were treated with iron-dextran (10 mg) or saline solution and 10 days later were infected with *L. infantum*. Mice were sacrificed two weeks after infection and the parasite load was determined in the liver and spleen.
Results: In both organs, iron overload decreased *L. infantum* growth in control mice but not in knock-out mice, indicating that the mechanism through which iron exerts its inhibitory effect on the growth of the parasite is dependent on the production of reactive oxygen species by the host cell.
Conclusion: Iron overload inhibits *L. infantum* growth in mice through the production of reactive oxygen species by NADPH oxidase, a potent antimicrobial mechanism to which *Leishmania* is susceptible.

P964 **Heat-shock response in *Brucella abortus***

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Objectives: Heat shock proteins are highly conserved molecules and serve a variety of functions in bacterial cells including protein folding and transport. They mainly serve to protect cells from various forms of stresses including heat shocks. In reference to the potent antigenicity of hsp and its activation of macrophages, the differences in virulence of *Brucella* cells may be originated from differences in patterns of response to heat shocks induced by the high degrees of fever that is usually manifested in brucellosis.
Methods: Five *B. abortus* strains, isolated from human and cows, were subjected to 39, 40, and 42 Celsius degrees heat treatments. The bacterial whole cell proteins were extracted and resolved by SDS-PAGE electrophoresis. Western blottings were used to detect antibody production against the extracted bacterial proteins especially hsp60 in both control and patient sera.
Results: SDS-PAGE gels revealed protein bands mainly in the range of 10–100 KDa. The amounts of a 60 KDa protein band (hsp60) was significantly enhanced following heat shocks in relation to the unheated bacterial cells. The heat shock response points to a significant higher production of a 60 KDa protein (hsp60). The sera from brucellosis patients reacted with several of these cell derived protein bands in western blots, none of which were reactive with sera from healthy individuals. The western blot protein bands showed striking differences.
Conclusion: The results of this study point to the immunogenic properties of *Brucella* heat shock proteins, especially the overwhelming

response towards hsp60. Therefore, hsp60 can be an appropriate antigenic candidate for engineering subunit vaccine against *Brucella*, as well as for ELISA test development.

P965 Deletion of a glycosyltransferase in *Enterococcus faecalis* leads to abolishment of glycolipid synthesis and increased susceptibility to opsonophagocytic killing

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Objective: Enterococci are generally considered a commensal of the human gastrointestinal tract but may also cause bacteraemia, urinary tract infections, abdominal infections and endocarditis. We have shown previously that lipoteichoic acid (LTA) is the target of opsonic antibodies against *Enterococcus faecalis*. Glycolipids are important in the maintenance of membrane elasticity and constitute the lipid moiety necessary for anchoring LTA in the cell membrane.
Methods: A non-polar deletion mutant was created in gene EF2890 of *E. faecalis* 12030 by targeted mutagenesis. Bacterial glycolipids were analysed by a modified Blight-Dyer extraction and detected by thin-layer chromatography. Cell lysates were characterised by western blot using rabbit antibodies raised against whole bacterial cells and purified LTA. An opsonophagocytic assay (OPA) was used to test the susceptibility of the wild-type strain and delta-EF2890 against rabbit antisera. The hydrophobicity was analysed by comparing distribution in hydrophilic and hydrophobic solvents. LTA was quantified by ELISA using antibodies raised against enterococcal LTA.
Results: Homology search revealed that EF 2890 shares high similarity with alMGS, a glycosyltransferase from Acheloplasma laidlawii, involved in the synthesis of monoglycosyldiacylglycerol (MGlcDAG). MGlcDAG is a major constituent of bacterial cell membranes. Wild type and delta-EF2890 displayed equivalent growth kinetics and stationary phase survival. Glycolipid analysis by TLC revealed that inactivation of EF2890 leads to a complete abolishment of glycolipid synthesis. The loss of glycolipids resulted in a retarded motility of LTA in SDS-PAGE. The content of LTA in the cell wall of the mutant strain was significantly higher than in the WT. Compared to the WT, EF2890 mutant was more susceptible to opsonophagocytic killing mediated by polyclonal rabbit antiserum raised *E. faecalis* 12030.
Conclusions: Deletion of the glycosyltransferase EF2890 resulted in complete abolishment of glycolipid synthesis. The absence of glycolipids leads to an increased retention of LTA on the cell wall and increased suscebtibility to antibody-mediated opsonic killing. Since we have shown previously that opsonophagocytic killing of *E. faecalis* 12030 is exclusively mediated by LTA-specific antibodies, our results suggest that an increased epitope denisity may facilitate the deposition of opsonic antibodies.

P966 *Acinetobacter baumannii* outer membrane protein A targets voltage-dependent anion channel and induces early-onset apoptosis and delayed-onset necrosis in dendritic cells

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Objectives: *Acinetobacter baumannii* outer membrane protein A (AbOmpA) is an important pathogen-associated molecular patterns that has been associated with host cell death. We investigated the dendriric cell (DC) death in response to AbOmpA and its implication of immune responses against *A. baumannii*.
Methods: Murine bone marrow-derived DCs were treated with recombinant AbOmpA. Subcellular localisation of AbOmpA was determined by confocal microscopy and immunoprecipitation. Molecular mechanisms of DC death were analysed by flow cytometry and Western blot assay. Wild-type *A. baumannii* and isogenic AbOmp (–) mutant strains were intraperitoneally infected in the mice.
Results: AbOmpA induces DC death, which started as apoptosis and later transformed to necrosis. AbOmpA targets mitochondria and interactes with voltage-dependent anion channel (VDAC). Apoptotic cell death is preceded by the transient elevation of mitochondrial transmembrane potential and an increase of intracellular reactive oxygen intermediates (ROIs). These events are followed by a loss of mitochondrial transmembrane potential, mitochondrial swelling, activation of caspases, and externalisation of phosphatidylserine. Both apoptosis and necrosis of AbOmpA-treated DCs are significantly inhibited by N-acetyl cysteine, suggesting that ROIs are responsible for the induction of apoptosis and necrosis. AbOmpA induces maturation of DCs, but ROIs produced by DCs impairs the cytokine production of T cells interacted with DCs and induces T cell killing. Wild-type *A. baumannii* impars maturation of DCs and results in high mortality in mouse model, whereas AbOmpA knock-out mutant induces maturation of DCs and no animals died.
Conclusion: Our study indicate that VDAC-targeting AbOmpA induces DC death and the subsequent dysfunction of T cells for the adaptive immunity. Thus, we propose mmune dysfunction mechanisms of *A. baumannii*-infected hosts regarding the mitochondrial targeting of bacterial proteins.

P967 Tolerability of N-chlorotaurine, a new endogenous antiseptic, in the bronchopulmonary system

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Objective: N-chlorotaurine, an important representative of the long-lived oxidants produced by human leukocytes, can be applied in human medicine as an endogenous antiseptic. This study was designed to evaluate the tolerability of inhalative N-chlorotaurine (NCT) in the pig model.
Methods: Anesthetised pigs inhaled test solutions of 1% NCT (n = 7), 5% NCT (n = 6), or 1% NCT plus 1% ammonium chloride (n = 6), and 0.9% saline solution as a control (n = 7), respectively. Applications were performed every hour within four hours, i.e. 4 inhalations in total, with 5 ml each. Lung function, blood oxygenation, and circulation were monitored. Pharmacokinetics were investigated by oxidation capacity in bronchial fluid and by determination of taurine and chloride in serum. One hour after the last dosing the animals were euthanised, and lung samples for histology were removed.
Results: Arterial pressure of oxygen (PaO2) decreased significantly over the observation period of 4 hours in all animals. Compared to saline, only 1% NCT + 1% NH4Cl led to significantly lower PaO2 values at the 4 hours measurement (62 mmHg ± 9.6 vs. 76 mmHg ± 9.2, p = 0.014). The corresponding increase in alveolo-arterial difference of oxygen partial pressure (AaDO2) was significantly higher only in the 1% NCT + 1% NH4Cl than in the control group ($P < 0.01$), too. Pulmonary artery pressure increased about 9.7 mmHg by 5% NCT, about 7.8 mmHg by 1% NCT + 1% NH4Cl ($P < 0.05$ versus control), about 4.3 mmHg by 1% NCT ($P > 0.05$ versus control), and about 6.7 mmHg by saline.
Histological investigations revealed inflammatory reactions, districts with atelectasia, districts with emphysema, and districts with fragmentation of fibers in both the test groups and in the saline group with no statistical difference.
There was no systemic resorption of NCT detectable. Local inactivation of NCT below detectable levels took place within 30 min. The concentration of NCT tolerated by A549 lung epithelial cells in vitro was 0.25–0.5 mM, which was similar to that known from other body cells and 25-fold higher than that of chloramine T.
Conclusion: The endogenous antiseptic NCT was well tolerated at a concentration of 1% upon inhalation in the pig model. Addition of ammonium chloride in high concentration provokes statistically significant impact on blood oxygenation, which would require adjustment of dose. These results are in accordance with previous clinical studies in man and animals.

Parasitology

P968 A new approach in the diagnosis of intestinal parasites

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Background: Twelve years ago molecular diagnostics for the differentiation of *E. histolytica* and *E. dispar* were implemented in Leiden and proved to be highly sensitive and specific. At that time, using PCR only as an additional technique to differentiate cysts and/or trophozoits that were seen by microscopy, it was never anticipated to use PCR as a first line diagnostic. During the following years the isolation of parasitic DNA from faecal samples and PCR techniques, were improved and simplified. Moreover the introduction of real-time PCR made it possible to multiplex different targets into one reaction. These new technical possibilities made it feasible to introduce PCR also for the detection of other intestinal parasites. The first multiplex real-time PCR for the simultaneous detection of the three most important diarrhoea causing protozoa and an internal control proved to be a sensitive and specific method for the detection of *E. histolytica*, *G. lamblia* and *Cryptosporidium* (HGC-PCR). Two studies were performed to define a diagnostic strategy for the implementation of molecular methods in the routine diagnosis of intestinal parasitic infections in general practice patients and in returning travellers and immigrants.
Methods: DNA (n = 350) from faecal samples originating from patients attending their GP with gastrointestinal complaints was tested retrospectively with HGC-PCR. In a second study, PCR analysis was performed on a weekly basis on faecal samples (n = 3591) from returning travellers using HGC-PCR and a Strongyloides PCR. In both studies PCR results were compared with microscopy and anamnestic data.
Results: In both studies, detection rates of the parasitic infections included in the PCR were increased significantly as compared with microscopy. In the GP patients no other pathogenic parasites were detected using microscopy and in the returning travellers additional parasites were found with microscopy in 55 cases only, especially in those travelling to high risk areas.
Conclusions: Multiplex real-time PCR offers a highly sensitive and specific diagnostic alternative for labour intensive microscopy in clinical laboratory practice. Additional diagnostic methods for the detection of parasitic infections that are not included as PCR target can be limited to a selected group of patients.

P969 Optimisation of a flow cytometry protocol for detection of *Encephalitozoon intestinalis*

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Microsporidia are spore-forming protozoa considered emerging pathogens in immunocompromised patients. *Encephalitozoon intestinalis* is one of the most important microsporidian human pathogen. The infectious form is a small spore, usually resistant to common water treatment. Its detection is very important but is difficult, time-consuming and requires specialised technicians; phase contrast and/or semiquantitative immunofluorescence-microscopy are the most common used techniques.
Objective: Our main objective was to develop and optimise a specific Flow Cytometric (FC) protocol for detection of *E. intestinalis* and to establish its detection limit; additionally, FC viability studies were undertaken, in order to evaluate the infectious risk.
Material and Methods: A known concentration of *E. intestinalis* spores (Waterborne Inc, USA) were stained with different concentrations of a Alexa Fluor 488 mouse monoclonal antibody (Microspor-FA, A700A488; Waterborne), at varied times (45 min, 1 h30m and 12 hours), at different temperatures (37°C and 25°C) and then analysed in a FACSCalibur cytometer (Becton Dickinson, Canada) at FL1 (525 nm – green fluorescence). Several dilutions (2×10^5 to 1×10^4 spores/ml) were afterwards stained with the optimised antibody concentration and analysed by FC for assessment of the detection limit. Cross-reactions were investigated using both prokaryotic (*Escherichia coli*, *Staphylococcus aureus*) and eukaryotic microorganisms (*Candida albicans* blastoconidia and *Cryptosporidium parvum* and *Giardia lamblia* (oo)cysts). Suspensions with dead spores were stained with 5 μg/ml of propidium iodide (PI, Sigma) (a marker of death with cell membrane lesion) following the staining with the specific fluorescent monoclonal antibody and analysed at FL3 (670 nm-red).
Results: The optimal specific-antibody concentration was 10 μg/ml, incubated overnight at 25°C, in the dark; it provided a clearly separated histogram from autofluorescence. A threshold limit of 5×10^4 spores/ml was established bellow that value, fluorescence was insufficient to allow discrimination. No cross-reactions occurred with bacteria, fungi or parasites. When using the two fluorescent probes simultaneously (specific-antibody and PI) dead spores showed double fluorescence (FL1 and FL3). A new detection method using a FC protocol is now available for the detection of *E. intestinalis*, allowing the simultaneous assessment of viability of the infective forms.

P970 Chagas' disease: evaluation of a rapid immunochromatographic assay for the detection of anti-*Trypanosoma cruzi* antibodies

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Objectives: Chagas disease, caused by *Trypanosoma cruzi*, affects 7.6 million of people in Latin America. A survey was carried out to assess its prevalence in two rural Bolivian communities (Bartolo, Chuquisaca Dep., and Casas Viejas, Santa Cruz Dep., Bolivia), and to evaluate the performance of Chagas Quick Test, a new immunochromatographic assay (ICT) to detect anti-*T. cruzi* antibodies.
Methods: 226 subjects were enrolled (121 males, 105 females); the infection was evaluated using a conventional *T. cruzi* IgG ELISA test, the above mentioned ICT, and the microscopic analysis of Giemsa stained thick smears. Individuals showing discordant ICT-ELISA results underwent molecular analyses. DNA was extracted from blood dried on filter paper using Chelex-100® method. *T. cruzi* nuclear DNA was amplified using primers TCZ1 and TCZ2 for the first reaction and TCZ3 and TCZ4 for the nested amplification. Amplicons were sequenced and subjected to GenBank to give the most likely identification.
Results: ELISA was positive in 142 people (62.8%); infection rate increased according to age from 15.3% in 1–9 years old individuals, to 100% in over sixties, independently by sex. No parasitemic individuals were found by hemoscopic analysis. A total of 159 subjects (70.3%) proved positive to ICT; all subjects positive to ELISA had a positive result (relative sensitivity 100%), with an intensely coloured test line. Moreover, 17 individuals negative to ELISA proved positive to ICT, with a very faint test line. The agreement between ELISA and ICT was 92.4% (209/226) (kappa 0.83). Molecular assays confirmed the infection in 4 serologically discordant subjects.
Conclusions: seroprevalence of *T. cruzi* infection in the study population is very high, and increases rapidly with the age, showing that parasite active transmission occurs universally. Negative results obtained by microscopy fit in with the low sensitivity of this diagnostic tool conditioned by the low parasitaemia in chronic infected people. Chagas Quick Test showed an almost perfect accordance with the conventional ELISA. PCR confirmed to be an useful complement to serological methods; it contributed to increase ELISA sensitivity and partially corroborated ICT results, supporting the advice that very faint test lines have to be considered as likely positive. Further investigations are needed to define the infectious status of subjects with discordant serology and negative PCR assay.

P971 Serological screening of Chagas' disease in pregnant immigrant women proceeding from endemic areas in Spain

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Objectives: Maternal-fetal transmission of *Trypanosoma cruzi* generally occurs in 2–12% of pregnant infected mothers. The arrival of pregnant women proceeding from endemic areas to non-endemic countries due to immigration phenomenon make possible the apparition of this diseases in this group of patients. The possibility of congenital infection in pregnant immigrant women in non-endemic areas has been studied only occasionally.
Methods: We determined anti-*T. cruzi* antibodies in all the pregnant immigrant women proceeding from endemic areas attending in obstetric service of Hospital Central de Asturias, a universitary hospital in the Northern of Spain, from 07/03/07 to 23/10/07. The ID-Chagas antibody test (Particle Gel Immuno Assay – PaGIA, DiaMed-ID –) was used as screening assay: red coloured polymer particles are sensitised with three different peptides representing antigenic sequences of *T. cruzi*.
Results: During the period of the study we screened 103 pregnant women (mean age 29 years, limits 17–43). The countries of origin were: Ecuador (32.0%), Colombia (17.5%), Brazil (15.5%), Paraguay (9.7%), Dominican Republic and in a minor percentage Venezuela (5.8%), Argentina (2.9%), Bolivia (2.9%), Cuba (1.9%) and Mexico (0.97%). Sixty patients lived in urban areas and 24 in rural areas. In 20 patients the characteristics of the living place was unknown. Only five patients lived in houses where the reproduction of triatomine bugs is possible. Two patients had family history of Chagas' disease. None patients presented positive antibodies anti-*Trypanosoma cruzi*.
Conclusions: Although the pregnant women arrival of endemic areas, no case of Chagas' disease was detected, probably due to the absence of epidemiological risk factors. Undoubtedly further studies of large samples are necessary to evaluate maternal transmission risk in immigrant population in Spain.

P972 Paediatric Chagas' disease in a non-endemic area

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Objectives: During last years, increasing migration from Central and South America, the endemic area of Chagas Disease, has introduced among our paediatric population some patients infected by *T. cruzi*, infected either in their origin country or in ours, via vertical transmission from their infected mothers. The objective of this study is to describe the cases of children with Chagas Disease attended at our Imported Pathology Unit during the last four years (2003–2007).
Methods: All patients coming from endemic areas and newborns from Chagas Disease seropositive pregnant women, were studied by two ELISA tests (Bioelisa Chagas BiokitR with recombinant antigens and in-house ELISA with complete antigens). Patients with available sample (59) were also screened by nested PCR (TCZ3/Z4). Confirmed cases received Benznidazol treatment (0.7 mg/Kg/day during 60 days).
Results: Screening was performed to 155 patients, aged one day to 13 years old (110 immigrants and 45 born in Spain). Ten of them were seroreactive in both ELISA tests (6.45%) and we had enough blood from five of them for PCR tests, which were positive for all samples. Vertical transmission was demonstrated in two children born in our country, one from a Bolivian mother and the other from an Argentina's one. The eight immigrated children were born in Bolivia. Follow up is in course in eight out of the ten seroreactive patients (two of them didn't continue attending our hospital) in order to establish the effectiveness of treatment. Negative PCR and decreasing specific antibodies have been demonstrated in all studied patients.
Discussion: Our results demonstrate a seroprevalence for *T. cruzi* infection of 7.2% (8/110) among the paediatric immigrated population coming from Latin America, and an incidence of congenital infection of 4.4% (2/45). Both percentages are high enough to encourage serologic screening for Chagas Disease to all children coming from Latin America, mainly from Bolivia. Post treatment serologic control must continue until antibodies level become negative. We have demonstrated titles decrease, but negativisation has not been assumed in any case.

P973 Chagas' disease among Latin American immigrants in Madrid. A serological survey

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Introduction: Chagas' disease, caused by infection with *Trypanosoma cruzi* is a significant health problem in Latin American, where an estimated sixteen to eighteen million people are infected. Outside of endemic regions, Chagas' disease may be transmitted through the transfusion of infected blood components, congenital infection and organ transplantation.
Objective: To determine the sero-prevalence of antibodies to *T. cruzi* in a community sample of Latin American immigrants to Madrid, Spain.
Methods: Eligible subjects were born in South America, Central America or in Mexico. Participants were attending a variety of medical clinics at the hospital Carlos III, Madrid, Spain. Serum samples were collected from January 2005 to November 2007. Antibodies to *Trypanosoma cruzi* were identified using a commercial screening EIA method (Biokit, Spain). Specimens that were repeatedly reactive by EIA were analysed by an indirect immunofluorescent assay, (Biocientífica S.A, Argentina)
Results: A total of 588 serum samples were studied. The geographic distribution of the patients was: 274 Ecuador (46.6%), 88 Colombia (15%), 82 Bolivia (14%), 31 Peru (5.3%), 28 Brazil (4.7%), 18 Dominican Republic (3%), 11 Venezuela (1.7%), 8 Argentina (1.4%), 8 Mexico (1.4%), 6 Chile (1%) and 34 other countries of Latin American (5.9%).
Thirty patients out of 588 (5.1%) were considered reactive for antibodies to *T. cruzi* by EIA. Indirect immnunofluorescent assay was performed in 26/30 reactive samples by EIA, being 20 positive.
Twenty five reactive samples corresponding to 82 patients (30.5%), coming from Bolivia, 3/274 (1.1%) from Ecuador, and 2/5 (40%) from Guatemala.
Conclusions: High rates of *T. cruzi* seropositive patients among Latin American immigrants underscore the importance of screening in this population to prevent transmission by blood transfusion or organ transplantation as well as congenital infection.
The Chagas antibodies EIA showed improved sensitivity over the Chagas indirect immnunofluorescent assay (IFA).

P974 Chagas' disease in Latin American immigrants in Valencia, Spain

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Introduction: Chagas Disease is caused by *Trypanosoma cruzi*. Approximately 70% to 80% of infected individuals remain in the indeterminate form throughout their lives, whereas as many as 20% to 30% develop into a cardiac form (arrhythmia, heart failure, ...) or a digestive form (megaesophagus, megacolon). The only treatment available nowadays in Spain is Benznidazole.
Objectives: To analyse epidemiological and clinical features of adults patients with Chagas Disease.
Methods: Since January 2005 until November 2007, we tested sera from Latin American adults coming to a Tropical Disease consultation, or travelers with an epidemiological risk for *Trypanosoma cruzi* infection. Immunological diagnosis was made using commercially available serological tests: Recombinant ELISA (BioElisa Chagas, Biokit S.A®, or Dade Behring®), and IFI (MarDxDiagnostic®).
Case definition: Any patient with epidemiological risk factors and two or more different serological tests positive. Clinical and epidemiological

review, physical examination, HIV serology, chest radiography, electrocardiography (ECG), echocardiography, and radiographic contrast study of esophagus and colon were performed.
Results: Thirty six cases of Chagas disease have been identified, all of them Latin American immigrants. Mean age 40.17 years (23–65). Gender: 75% women, 25% men. Polymerase chain reaction was available in 20, and positive only in 2. Most of the cases (86%) were from Bolivia. Twenty nine (80%) were indeterminate forms, 4 patients (11%) had a chronic cardiac form, 2 patients (5%) a mixed form, and 1 (2%) a chronic digestive form. A forty four years old woman died because of dilated cardiomyopathy and complex ventricular arrhythmias. The other abnormal ECG findings were sinusal bradycardia, right bundle-branch block, complete A-V block, left anterior fascicular block. All patients were HIV seronegative. Treatment with Benznidazol was started in 8 patients.
Conclusion: Chagas Disease is an emergent disease in Europe because migratory movements. Most of our patients are asymptomatic women from Bolivia, however, about 20% of the patients have chronic forms that can progress to heart failure, and can require intensive and multidisciplinary care.

P975 Prevalence of oral trichomoniasis in patients with periodontitis and gingivitis using PCR and Direct Smear

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Objective: *Trichomonas tenax*, a commensal flagellated protozoan, inhabits in human oral cavity. This parasite is cosmopolitan and frequently found in patients with poor oral hygiene and advanced periodontal disease. There is only one published study that rebound the prevalence of this parasite in Iran. This PCR based study compared the prevalence of oral trichomoniasis in patients with oral diseases and a healthy control group.
Methods: From May 2006 to April 2007, the subgingival dental plaques of 160 patients with gingivitis or periodontitis and 160 controls who attended to Dental School of Shahid Beheshti Medical University, Iran were taken and examined by wet mount smear, and Giemsa staining. Likewise, a PCR protocol was developed for specific detection of *T. tenax* using a pair of primers designed for its 18S rRNA gene.
Results: Thirty three (20.6%) of patients were PCR positive while 28 (15.5%) were diagnosed using wet preparation and Giemsa staining. In the other hand, 2 (1.9%) of control group were identified positive by PCR procedure. The prevalence of oral trichomoniasis in our study (20.6%) was compatible with many other published reports which mostly has ranged from 12%-32%.
Conclusion: The study revealed dependence between the frequency of occurrence of *T. tenax* and the state of periodontitis. The present PCR procedure could provide a simple and rapid detection method of *T. tenax* in dental plaque.

P976 Real-time PCR in diagnostics of *Trichomonas vaginalis* infection

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Objective: *Trichomonas vaginalis* is the most common parasitic cause of sexually transmitted infection in the world. Methods used for the laboratory diagnosis of *T. vaginalis* infection include microscopic examination of wet mount preparations, culture and more recently polymerase chain reactions (PCR). The objective of this study was to determine the prevalence of *T. vaginalis* infection in men tested at the Parasitology department of the Croatian National Institute of Public Heatlh between July and October 2007. A comparison of real-time PCR with culture and wet-mount microscopy in the diagnosis of trichomonosis was also performed.
Methods: Urine samples from 151 men were tested for *T. vaginalis* using culture and real-time PCR. One part of urine sediment was inoculated in Diamond's medium and examined for motile organisms after 24, 48 and 72 hours. From other part of sediment DNA was isolated using a spin column kit (High Pure PCR Template Preparation Kit; Roche Diagnostics GmbH). Real-time PCR was applied for detection of *T. vaginalis* DNA using a TaqMan Universal PCR Master Mix kit (Applied Biosystems). To rule out the presence of PCR inhibitors in the individual patient specimens, an internal control kit (TaqMan Exogenous Internal Positive Control Reagents; Applied Biosystems) was used. The amplification and detection were performed using 7500 Real Time PCR System machine (Applied Biosystems).
Results: Out of the 151 urine samples tested, 2 were positive by culture and 2 more (4 altogether) by PCR. The prevalence of *T. vaginalis* infection in male study population according to PCR was 3%. Real-time PCR also exhibited greater sensitivity than culture did (100 vs. 50%).
Conclusions: Despite all the efforts invested in development of better diagnostic tests for trichomonosis, PCR is not commonly used. The main reason is that *T. vaginalis* infection is not perceived as a significant public health problem. However, this perception should be changed based on recent studies suggesting a role of *T. vaginalis* in adverse pregnancy outcome and HIV transmission. A real-time PCR has immediate and important implications for diagnostic testing in the microbiology laboratory. The enhanced sensitivity, ease of performance and speed of this technology make it an appealing alternative to conventional methods which have been used for many years.

P977 Prevalence of hydatid cyst in a slaughter-house in Iran: a ten-year survey

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Objectives: Parasitic diseases are a major group in zoonosis, in which hydatidosis has a special situation prevalence of hydatid cyst in a region's animals in dangerous that could lead to prevalence of the disease in man. This will also increase inurn, disadvantages such as removing, contaminated organs of animals (e. g. liver and lung), decrease products like milk, weigh, wool, quality of meat plus economic expenses. Evaluation and determining of health and economic importance of each disease would be the first step in any prevention and control programs.
Methods: In this retrospective survey, slaughtered animals (cow, sheep) were studied in respect to hydatid cyst infection according to kind of animal, year, and season in a ten-year period (1996–2005) in Semnan's slaughter-house.
Results: The findings of this study showed that 13–25% liver and 15.97% lung were confiscated (the total no. of slaughtered animals was 359716 include 39698 cow and 320018 sheep). Based on the results 37.43% of livers and 51.18% lungs were infected by hydatid cyst. The mean of liver and lung infection in cow was 4.84% and 4.41% respectively. This rate was 5.05% and 6.84% in sheep. On the basis of season the most infection rates in both animals were observed in turn in autumn and winter. The estimated economic damage due to omission of infected organs, only by hydatidosis in this period was 1667720000 Rails.
Conclusion: The results of the study, from the epidemiological aspect, could be a confident starting point, in preventive and controlling programs of this important parasitic disease. Due to zoonosis the presented proposals would decrease the human hydatidosis.

P978 Bursts of trichinellosis in a Romanian industrial area

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Objectives: To overview and analyse epidemiologically all the trichinellosis cases diagnosed during an endemic decade in an industrial area from Hunedoara County, situated in the central-western part of Romania.
Methods: We retrospectively extracted data from the medical records of 353 patients hospitalised during the period 1996–2005 at "Dr. Al. Simionescu" Hospital from Hunedoara, Department of Infectious Diseases. There have been analysed the following particularities

regarding the study group: age, gender, inhabitance area, year and season of hospitalisation and profession.
Results: Most of the patients (27.47%, n = 37) were in the age group of 10–19 years old; 198 patients (56.09%) were women; 233 patients (66.01%) lived in the urban areas among whom 195 patients were inhabitants of Hunedoara town; 1997 was the year with the largest number of the cases (32.58%, n = 315); 55.24% of the patients were hospitalised during the winter; 42.66% were unemployed people.
Conclusion: Hunedoara town and the surroundings belong to a heavy industrial area in decline where a lot of people are poor and unemployed. There is also a large gipsy community living in unsanitary conditions, eating uninspected pork products. This facts can explain the frequent bursts of trichinellosis that occur in this area especially within the group mentioned above. Most of the cases were diagnosed in winter when, according to regional food habits, a lot of pigs are slaughtered.

P979 Subtyping *Blastocystis hominis*: is subtype 2 responsible for asymptomatic infections?

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Objectives: *Blastocystis hominis* is probably the most common eukaryotic organism in the intestinal tract of humans, with increasing reports indicating its presence in patients with gastrointestinal and dermatological manifestations. However, little is currently known about its pathogenesis in human infections. Isolated both from humans and various animals, this interesting protozoon has numerous known subtypes and the relation between these subtypes and its pathogenesis is yet under research. The aims of this study were to identify *B. hominis* isolates in both adult and paediatric patients with and without symptoms in two hospitals in Manisa City, Turkey, and subtype them to identify any correlation between the reported symptoms and subtypes.
Methods: Stool samples of the individuals were examined with routine O&P examination and the presence of *B. hominis* was confirmed with the examination of trichrome stained smears. All positive samples were inoculated in Robinson's medium and a total of 92 samples were included in the study. The individuals were grouped according to having any related symptoms or not. DNA was extracted from each sample using the QIAamp DNA Stool Mini Kit (QIAGEN, Hilden, Germany)according to the recommendations of the manufacturer, followed by genotyping with PCR using seven different primers. Chi-square testing was applied for the statistical analyses.
Results: Microscopic examination of the stool samples of symptomatic cases revealed that only 11 (18.6%) were *B. hominis*-positive. Subtype 3 was by far the most common (n = 31; 55.4%) *Blastocystis* subtype and Subtype 2 was found more common in asymptomatic cases with a statistical significance ($p<0.05$). No other significant relation was detected with other subtypes in the study.
Conclusion: Concordant with the results of the previous studies, Subtype 3 isolates were found to be most common in Manisa, as well. Further studies conducted with cases from different regions are needed to confirm the present relation with Subtype 2 and asymptomatic infections.

P980 Prevalence of *Giardia intestinalis lamblia* among children with allergies from Saint-Petersburg, Russia

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Odjectives: The percentage of allergic children in Russian megalopolis is increasing each year. It's considered that about 70% of children in Saint-Petersburg have various kinds of allergies.. From the previous studies we see that there are certain association between the *Giardia intestinalis* detection in stool specimens and clinical manifestations of allergies. In Saint-Petersburg 43–45% of children with allergy symptoms from atopic dermatitis to bronchial asthma were infected with *Giardia intestinalis*. However the possibility that allergies might be associated with other parasites (*Ascaris lumbricosis*, *Eterobius vermicularis*, *Trichinella spiralis*, *Dirofilaria immitis*) was not investigated. Therefore the goal of this study is to find connections between allergy symptoms, *Giardia intestinalis* and helminthes.
Materials and Methods: 40 children under 12 years old with allergy were involved in this study. 20 of them were positive for *Giardia intestinalis* and 20 were negative. For *Giardia intestinalis* detection we used traditional microscopy and PCR.
Feaces from all children were examined by PCR and microscopy for helminthes identification.
DNA from faeces were extracted with "DNA-express" kit (Lytech, Russia). PCR was performed with specific primers, and detected by gel-electrophoresis in 1.5% agarose.
Result: In all 40 cases microscopy analysis was negative for helminthes and for *Giardia intestinalis*. Patients with *G. intestinalis* group have been tested positive by PCR at least twice.
In *G. intestinalis* positive group (20 children) 6 patients were detected with *T. spiralis* (30%), 5 with *A. lumbricosis* (25%), three cases with *D. immitis* (15%) and 2 cases with *E. vermicularis* (10%).
In *G. intestinalis* negative group (20 children) one was detected with *T. spiralis* (5%), 3 – *D. immitis* (15%). *A. lumbricosis* and *E. vermicularis* were not detected.
Conclusion:

1. Children infected with *Giardia intestinalis* are more predisposed to helminthes contamination.
2. Prevalence of *Trichinella spiralis*, *Ascaris lumbricosis*, *Dirofilaria immitis*, *Eterobius vermicularis* among allergic children was high.
3. The only helmint that was found in same percentage from both groups of children was *Dirofilaria immitis*. The only helminthes that is not transmitted through the intestinal tract.

P981 Preliminary analysis of the molecular phylogenetics of *Toxocara canis* (Nematoda: Ascaridoidea) using nuclear ribosomal second internal transcribed spacer sequences

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Objectives: Toxocariasis is a zoonotic disease caused by *Toxocara canis* and *T. cati*. Human is their paratenic host. Although many clinico-epidemiological aspects have been studied in almost 60 years after the discovery of human disease, many biological and molecular aspects are still lack of knowledge and research. After a careful review of literature we did not found any study describing the phylogenetics of *Toxocara* using any molecular marker.
Methods: For these reasons, nuclear internal transcribed spacer 2 (ITS2) rDNA sequences were used for the first molecular phylogenetic analysis and tree of five *Toxocara* species. ITS2 rDNA sequences were already available from GenBank (accession numbers AB110034, AB110033, EU189085, AM231609 and AB027152), corresponding to *T. canis*, *T. cati*, *T. vitulorum*, *T. malaysiensis* and *T. tanuki*. Phylogenetic analysis was made after aligned these sequences using GeneDoc 2.7 (Figure), using the method of Maximum Parsimony and the bootstrap test for phylogeny with MEGA 4.
Results: As expected, *T. canis* and *T. cati* ITS2 alleles are monophyletic, as well *T. malaysiensis* with *T. vitulorum* (Figure). As expected also, we observed a high nucleotide diversity (p = 0.705825; Tajima test = 0.461359) between the studied species.
Conclusions: The nuclear ITS rDNA sequence has been extensively used to define genetic markers for different species of nematodes and the aims of this study were to use the ITS2 to investigate the levels of genetic variation within *Toxocara* and generate a first phylogenetic tree (Figure). If there are normal levels of gene flow between populations (or species), these populations are likely to be similar in their overall genetic characteristics, and genes for important characteristics are likely to spread easily. ITS2 of other *Toxocara* species should be included in further analysis (eg. *T. genettae*, *T. lyncis*, *T. vincenti*, among others), to

better define the evolutionary relationships and phylogeny of the genus *Toxocara*.

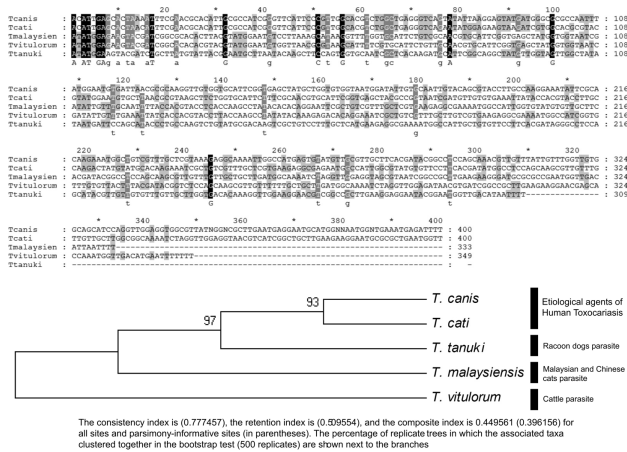

Figure. Multiple sequences alignment (upper) and evolutionary relationships of *T. canis* and other spp. (lower). The consistency index is (0.0777457), the retention index is (0.509554), and the composite index is 0.449561 (0.396156) for all sites and parsimony-informative sites (in parentheses). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches.

P982 Is multiple-stool-specimen ova and parasite examination necessary?

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Objectives: We performed a retrospective analysis of 12,862 ova and parasite (OP) examinations of stool specimens from 8109 patients visited at Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, between 2004 and 2006 to evaluate the utility of such tests in multiple, independently-collected stool specimens.

Methods: Multiple-specimens from a single patient were considered related to the same clinical episode if collected within 14 days. A series was thus defined as specimens of the same clinical evaluation. A positive result was defined as the presence of pathogenic OP in stool specimen. Presence of *Trichomonas hominis*, *Chilomastix mesnili*, *Endolimax nana* and *Entamoeba coli* were also considered positive. A second or third specimen was considered positive for a parasite only if the previous specimens were negative. As a positive result for another parasite in the same series was considered a further positive result, a single series could have more than one positive result. Standard parasitological techniques were used.

Results: Of the 12,862 specimens submitted, 10,086 corresponded to single-specimen series, 1942 to 2-specimen series and 734 to 3 or more specimen series. To evaluate the utility of multiple-specimen examination we focused on series of at least 3 specimens. The first specimen collected was diagnostic in 49.15% (29 of 59) evaluated cases. The examination of 2 specimens increased sensitivity to 79.66% (47 of 59). The third specimen collected provided additional information in 12 cases (20.34%). Information provided by the first specimen, in comparison with the second, was not statistically significant ($p = 0.074$), although a tendency was observed. Differences between information provided by the first and third specimen were statistically significant (0.006). Differences between second and third specimens were not statistically significant ($p = 0.352$).

Conclusions: These data suggest that 2 specimens per series should be studied in order to achieve sensitivity closest to 80%. As the difference in information provided by the second and third specimens is not significant, a third specimen is not needed.

P983 Anti-giardial activity of carvacrol, thymol and eugenol

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The protozoan parasite *Giardia lamblia* is recognised as one of the most important non-viral infectious agents causing diarrhoeal illness in humans around the world. Current therapy against *G. lamblia* infection (giardiasis) is unsatisfactory due to high incidence of undesirable side effects and a significant failure in clearing parasites from the gastrointestinal tract. Consequently, new compounds are being screened for antigiardial activity, namely essential oils and their pure compounds, which revealed on previous studies important antibacterial, antifungal and antiparasitic activity. In the present work, we studied the effects of pure phenolic-compounds such us carvacrol, eugenol and thymol on *G. lamblia* growth and adherence. Culture trophozoites of WB strain (ATCC 30957) were incubated in growth medium with different concentrations of phenolic compounds for 48 h at 37 °C under anaerobic conditions. The concentrations that inhibit the growth at 50% (IC50) were determined by total cell counting. To study the effects on *Giardia* adherence, an inoculum of 5×10^4 trophozoites was exposed to pure phenolic-compounds at IC50 concentrations during 7 h at 37°C in Keister medium without serum. The number of unattached and attached cells was determined microscopically using a haemocytometer (Neubaeur cell counter chamber) and the results were expressed as percentage of attached trophozoites in relation to the total number of *Giardia* cells reached from each assay. The experiments were performed in duplicate and in three independent assays ($n = 3$), and the analysis of variance (ANOVA) was used. The growth of *G. lamblia* was significantly inhibited by thymol, carvacrol and eugenol. The most active compound was thymol (IC50 = 47 μg/ml), followed by carvacrol (IC50 = 50 μg/ml) and eugenol (IC50 = 131 μg/ml). The tested phenolic compounds inhibit *G. lamblia* trophozoites adherence ($p<0.001$) from the first hour of incubation. The total cell number (attached and unattached cells) after 7 hours of incubation was unaffected by eugenol, but was decreased by carvacrol (30%, $p>0.05$) and thymol (50%, $p<0.01$). These results suggest that carvacrol, thymol and eugenol have antigiardial activity inhibiting the cells growth and attachment, inducing cell death. Therefore, these compounds seem to have potential for use as therapeutic agents against giardiasis.

This work was supported FCT POCTI (FEDER).

P984 Effects of phenolic-rich essential oils on the morphology and adherence of *Giardia lamblia* trophozoites

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Attachment of *Giardia lamblia* trophozoites to enterocytes is essential for colonisation of the small intestine and is considered a prerequisite for parasite-induced enterocyte dysfunction and clinical disease. *G. lamblia* trophozoites can also attach to in vitro cultured cells as well as to glass, plastic and to a variety of artificial substrates. Considering that inhibition of its attachment may have therapeutic potential, it was of interest to study the ability of essential oils to inhibit the *G. lamblia* adherence.

In previously studies we have showed that phenolic rich essential oils inhibit the growth of *G. lamblia* in vitro. In the present work, we evaluated the effects of essential oils on morphology and adherence of *G. lamblia* trophozoites. Essential oils were obtained by hydrodistillation from fresh plant material and analysed by GC and GC-MS. Constituents were identified from their retention indices on two different phases GC columns (polydimethylsiloxane and polyethyleneglycol) and from their mass spectra, which were compared with reference data. An inoculum of 5×10^4 trophozoites (WB strain, ATCC 30957) was exposed to essential oils at concentrations that inhibit the growth at 50% (IC50) during 7 h at 37 °C. The morphological alterations were studied by optical and transmission electronic microscopy. The number of unattached and attached cells was determined microscopically using a haemocytometer and the results were expressed as percentage of attached trophozoites in

relation to the total number of *Giardia* cells reached from each assay. The experiments were performed in duplicate and in three independent assays (n = 3), and statistically, the analysis of variance (ANOVA) was used.
The essentials oils from *Thymus zygis* ssp. *sylvestris*, *Thymbra capitata*, *Origanum virens*, *Lippia graveolens* and *Syzygium aromaticum* inhibit *G. lamblia* trophozoites adherence (p<0.001) since the first hour of incubation. The main morphological alterations promoted by essential oils were deformations in typical trophozoite appearance, such as modifications on the cell shape, alterations in plasma membrane, presence of precipitates in the cytoplasm and intracellular clearing. The results suggest that these phenolic-rich essentials oils have anti-giardial activity modifying the attachment ability and promoting cell death by a lyses process, having potential for use as therapeutic agents against giardiasis.
This work was supported FCT POCTI (FEDER).

P985 High Prevalence of *Giardia* in dogs from Portugal: genetic characterisation of isolates by sequencing analysis of beta-giardin gene locus

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Human giardiasis is considered a zoonotic infection, although the role of animals in the transmission to humans is still unclear. Molecular characterisation of cysts of human and animal origin represents an objective means to validate or reject this hypothesis.
Some animal derived isolates appear to be similar or identical to human derived genotypes while others represent unique genotypes that seem to be host-specific. When considering the zoonotic potential of each genotype, the Assemblage A and B are believed to represent a major risk for human health since both appear to be infective to a wide range of wild and domestic host species. In the present study, isolates of *Giardia* were genetically characterised by using PCR-based procedures to determine the occurrence of potentially zoonotic genotypes of *G. lamblia*.
Faecal samples (n = 37) were obtained from dogs in kennel situations in north of Portugal. The parasitological study was performed by microscopy observation (direct examination and after concentration by Copropack S.A.F, Biomedics, S.L.) and *G. lamblia* coproantigen detection was performed by immunocromatografic method (Sick *Giardia*/simple *Giardia*, Operon). The β-giardin gene locus was amplified by nested-PCR and PCR products were sequenced with dRodamina Terminator Cycle Sequencing kit. The reading was performed using an ABI PRISM 310 automatic DNA Sequencer and the obtained sequences were aligned with those available in Genbank using Clustal X program. Sequence accuracy was confirmed by two-directional sequencing.
A total of fourteen faecal samples were positive to *Giardia* cysts (51.8%), one for Isospora sp. (3.7%), four to *Ancylostoma* sp. (14.8%), two to *Toxocara canis* (7.5%) and one to *Capillaria* spp. (3.7%). The *Giardia* antigen detection test was positive for 13 dog faeces samples (48%). Of 16 *Giardia* cysts and/or antigen positive faeces, 12 samples were successfully amplified at the beta-giardin locus gene. The sequencing analysis showed that the assemblage A was detected in eight samples. This is the first genetic characterisation of *Giardia* isolates in dogs from Portugal. The results confirmed the high prevalence of *Giardia* in dogs and showed that the zoonotic Assemblage A was common in this domestic animal. The results demonstrate that a significant proportion of dogs in north of Portugal harbour zoonotic *Giardia* and should thus be considered a potential reservoir for infection in humans.
This work was supported FCT POCTI (FEDER).

P986 Albendazole versus praziquantel in the treatment of neurocysticercosis: a meta-analysis of comparative trials

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Objective: We investigated the role of albendazole and praziquantel in the treatment of patients with parenchymal neurocysticercosis by performing a meta-analysis of comparative trials of their effectiveness and safety.
Methods: We performed a search in the PubMed database, Cochrane Database of Controlled Trials, and in references of relevant articles. Six studies were included in the meta-analysis.
Results: Albendazole was associated with better control of seizures in comparison with praziquantel in the pooled data analysis using a random effects regression model of the incidence of seizure control [patients without seizures/(patients X years at risk)] [156 patients in 4 studies, point effect estimate =4.94, 95% confidence interval 2.45–9.98]. In addition, albendazole was associated with better effectiveness regarding the total disappearance of cysts in comparison with praziquantel (335 patients in 6 studies, random effects model, OR=2.30, 95% CI 1.06–5.00). There was no difference between albendazole and praziquantel regarding adverse events and development of intracranial hypertension due to the administered therapy.
Conclusion: A critical review of the available data from comparative trials suggest that albendazole is more effective than praziquantel regarding clinically important outcomes in patients with neurocysticercosis. Nevertheless, given the relative scarcity of trials, more comparative interventional studies, especially randomised controlled trials, are required to draw a safe conclusion about the best regimen for the treatment of patients with parenchymal neurocysticercosis.

P987 Assessment of parasitic and microbial infections in vegetables using different washing methods

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Objectives: Vegetables are very important part of the dairy food. Contamination can occur in vegetables particularly if there is a poor sanitation condition such as what in present in some part of Ilam province in Iran. Both microbial and parasitic infection can be found on vegetables if the washing method is not accurate.
Methods: 180 vegetable samples were randomly selected from retailer in different areas of the Ilam city. Vegetables were rinsed with tap water thoroughly keeping the water for examination while the sample treated with detergent and hypochlorite separately and the water kept again for examination purposes. Formalin-ether concentration was applied comparing to sedimentation and direct microscopy
Results: The parasitic infections were observed in more than 40% of samples while the microbial infection in more than 70%. The parasitic infections were reduced after treating with detergent but this was not statistically significant. No significant decrease was observed for the microbial infection after washing with soap while it was significant after treating with hypochlorite. *Giardia lamblia* (43%), *Ascaris lumbricoides* (5.6%), *Taenia* (8%), *Entamoeba coli* (7.8%), other Amoeba (7%) and *Trichucephalus* (5%) were the most prevalent infection in samples.

P988 Outbreak of cyclosporiasis in Istanbul, Turkey, during an extremely dry and warm summer

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Objectives: *Cyclospora cayetanensis* is an important agent of traveler diarrhoea in developed countries and was responsible for numerous foodborne outbreaks worldwide. We describe a *Cyclospora* outbreak in a region between Istanbul and Kocaeli during a very warm and waterless summer.
Methods: 286 patients with diarrhoea were admitted to Anadolu Medical Center outpatient clinics during July-August 2007. Stools are investigated routinely for coccidian parasites in the microbiology laboratory of the Anadolu Medical Center, especially in the summer months. Modified acid-fast stain and careful microscopic examination (including UV light analysis) are performed after Para Pak (Meridian Diagnostics) concentration. *C. cayetanensis* specific polymerase chain reaction (PCR) based on nucleotide sequences for ITS-1 region was performed in microscopy positive stools.

Results: *C. cayetanensis* oocysts were found to be positive in the stool of 16 patients. *C. cayetanensis* (7 female, 9 male, median age is 41.94 plus/minus 10.0). *C. cayetanensis* DNA were found to be positive by PCR in only 12. The diarrhoea was persistant in most of these patients. No cases were detected in June and September.
Conclusion: *Cyclospora* prevalence was significantly higher compared with 2006 season (data not shown). The food histories showed that some patients had eaten a green salad. There were additional cases that we could not have the possibility to further analyse. All the patients were successfuly treated with trimetoprim-sulfametoxazol (TMP/SMX 160/800 mg bid). *C. cayetanensis* infection is more prevalent in Turkey during summer as reported previously. The laboratories should always consider performing modified acid-fast staining (very efficient for this purpose) to diagnose this parasite in acute or sub-acute diarrhoea cases. A single-step PCR is not as sensitive as the conventional method but serve to further analyse the genome of the parasite. An extremely dry summer came after an extremely warm winter in 2006–2007 season. This deserves further speculation to correlate with outbreaks.

P989 *Fusarium oxysporum* and free-living amoebae

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Objectives: *Fusarium oxysporum* is responsible for infections in immunocompromised patients. Some authors have identified hospital water distribution systems as reservoirs of *Fusarium* species. In these systems, amoebae of *Acanthamoeba* and *Hartmannella* genus are also often recovered and it is known that these protozoa can influence the growth of other microorganisms as bacteria or yeasts. We have investigated the potential interactions between *A. castellanii* or *H. vermiformis* and *F. oxysporum* (isolated from a cornea).
Methods: *A. castellanii* ATCC 30234 or *H. vermiformis* ATCC 50256 trophozoites (5.10^5/ml) were incubated with 5.10^4/ml conidia of *F. oxysporum* (MOI = 0.1). The coculture was incubated in PBS at 27°C during 24, 48 or 72 h. Controls were realised by incubating fungus in PBS alone. After incubation and amoebae lysis, dilutions of this lysate were plated on Sabouraud agar to determine CFU.
Trophozoites (5.10^5/ml) of each amoebae strain were incubated during 24, 48 or 72 h in PBS at 27°C. Amoebae were pelleted and *F. oxysporum* conidia (5.10^4/ml) were cultivated in the resultant supernatant. After 24 or 48 h of incubation, each suspension was plated on Sabouraud agar and CFU were numbered. The germinative potential of *F. oxysporum* conidia was microscopically determined at 4, 8, and 24 h.
Results: In cocultivation experiment, the presence of each free living amoeba led to an increase of *F. oxysporum* growth whatever the duration of incubation.
The contact (24 or 48 h) of *F. oxysporum* with the amoebae supernatants promoted an increase of the number of fungal CFU, compared to *Fusarium* growth in PBS alone. The conidia germination, microscopically verified, occurred only with the supernatant of *H. vermiformis* after 24 h of incubation. No germination was observed after incubation in PBS or in *A. castellanii* supernatants.
Conclusion: The results of this preliminary study indicate that the presence of free living amoebae in the same environment could lead to an increase of the *F. oxysporum* development. Moreover, the *A. castellanii* and *H. vermiformis* trophozoites used here can release substances able to favour the growth of this fungus. So, in hospital water taps, a special attention should be paid to the presence of free living amoebae, which can not only promote bacterial growth, but also development of fungal pathogens.

Molecular diagnosis of viruses

P990 Contribution of RSV and hMPV to patients with influenza-like infections during 2006–2007 season in northern Greece

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Background: Every year, during influenza season, many different viruses (such as RSV and the newly discovered hMPV) co-circulate, infecting individuals in the community.
Objectives: The aim of this study was to determine the contribution of RSV and hMPV to influenza-like illness (ILI) cases during 2006–2007 influenza season in N.Greece.
Materials and Methods: One hundred and twenty-nine pharyngeal swabs (129) from patients presented as ILI infections during the last influenza season (2006–2007) were examined at the National Influenza Centre by conventional and molecular techniques for influenza viruses, RSV and hMPV.
Results: Of the 129 patients examined, 52 (40.3%) were laboratory confirmed influenza (AH3) infections, 7 (5.4%) were laboratory confirmed RSV infections and 8 (6.2%) were laboratory confirmed hMPV infections. Five of the seven RSV cases appeared in children younger than 3 years old. Co-infections (RSV and A(H3) influenza viruses) were detected in two cases, concerning adults. From the 8 hMPV cases, five were children <9 years old and three were young adults (<25 years old). Co-infections (hMPV and A(H3) influenza viruses) were detected in two cases, concerning both children and adults. 2006–2007 influenza season started in January 2007 and lasted till the end of March, peaking in February. RSV was detected in the beginning and the end of this season, while hMPV was circulating during the whole period.
Conclusions: As RSV and hMPV infections mimic influenza, it is common to be clinically confused, especially in children. Including RSV and hMPV in the laboratory diagnosis of ILI cases distinguish the contribution of these viruses in such infections.

P991 Multiplex MLPA assay for simultaneous detection of 6 viral agents causing central nervous system infections

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Objectives: Molecular DNA-based diagnostics are increasingly being used for detection of infectious agents in cerebrospinal fluids. However, the increasing list of targets and the small sample volume are challenges that demand an improved molecular diagnostic approach. Therefore, in this study we have developed a multiplex ligation-dependent probe amplification (MLPA) assay for the simultaneous detection of Cytomegalovirus, Epstein Barr virus, Herpes Simplex virus I and II, Varicella Zoster virus, enteroviruses and an internal control.
Methods: An MLPA reaction consists of three steps: first an annealing step where the 2 probes hybridise to their target region, secondly a ligation step to link the two probes, and lastly a PCR to amplify the ligated probes followed by detection by gel electrophoresis. The main advantage of using MLPA is that only one primer set is used for the final amplification reaction, in contrast to for example multiplex PCRs. This offers the opportunity to detect up to 45 targets simultaneously without affecting the reaction negatively. The MLPA reaction is preceded by a reverse transcriptase PCR to ensure the detection of both RNA and DNA viruses. In this study the MLPA assay was tested on 50 cerebrospinal fluids of patients with suspected central nervous system infection. The MLPA was performed in two centres and compared with singleplex real-time PCR analysis performed in routine diagnostic laboratories. Real-time PCR analysis were considered to be the "gold standard".
Results: MLPA results showed 100% accordance (intra-laboratory variation) and a repeatability of 92% (inter-laboratory variation). Overall the MLPA test had a inter-laboratory sensitivity of 87% (number of correct positives by MLPA/ total number of positives by real-time PCR)

and a inter-laboratory specificity of 94% (number of correct negatives by MLPA/ total number of negatives by real-time PCR). When analysed per target individually, the inter-laboratory sensitivities were between 90 and 100% except for CMV, where no positive samples were tested, and EBV, where no positive sample could be identified. The inter-laboratories specificities per target varied between 98% and 100%.

Conclusions: In conclusion, although further optimisation is needed to improve the EBV detection and CMV positive samples should be tested, this MLPA assay is a new promising tool in the field of rapid simultaneous detection of infectious agents causing central nervous system infections.

P992 Performance of the APTIMA HPV assay as compared to Hybrid Capture-II on cytologically and histologically defined specimens

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Objectives: The APTIMA HPV Assay detects HPV E6/E7 mRNA from 14 high-risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) in liquid Pap specimens. It utilises Gen-Probe's target capture, Transcription-Mediated Amplification, and Hybridisation Protection Assay technologies, incorporates an internal control, and can be run on both the semi-automated Direct Tube Sampling (DTS) instruments and the fully automated TIGRIS DTS System (TIGRIS). In this study, the performance of the APTIMA assay was evaluated in residual Cytyc PreservCyt liquid-based cytology samples from US and France referral populations, including samples determined to contain atypical squamous cells (ASC), and samples classified as CIN 1–3.

Methods: PreservCyt samples (n = 367) were diluted 1/4 in APTIMA specimen transport media and tested on TIGRIS. Samples were also tested using the Digene Hybrid Capture 2 (HC2) assay following its standard assay procedure.

Results: The APTIMA assay detected 259 of the 367 samples tested (70.6%) and the HC2 assay detected 260 (70.8%). The reactivity rate for the APTIMA assay in ASC samples was 69.1%. For these same samples the HC2 assay reactivity rate was 81.8%. For samples determined to be CIN-1, -2, or -3 (n = 375), APTIMA assay reactivity rates were 76.8%, 95.5%, and 96%, respectively, while HC2 assay reactivity rates were 76.8%, 93.2%, and 92%, respectively. For samples determined to be CIN 2 or -3 (n = 319), the APTIMA assay achieved 95.8% reactivity, while HC2 assay reactivity was 92.4%. Inter-assay concordance was 87.5% overall, 80% for the ASC samples, and 95% for the CIN-2 or -3 samples.

Conclusion: The APTIMA HPV assay exhibited lower reactivity than the HC2 assay in samples with ASC cytology, and higher reactivity than the HC2 assay in disease-positive (CIN-2 or -3) samples. Concordance between the APTIMA and HC2 assays was 95% in histologically disease-positive specimens.

P993 Analysis of VZV and HSV in different matrices by the use of affigene kits

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To date there is no consensus on how to best diagnosing for varicella zoster virus (VZV) and herpes simplex virus (HSV), which sample matrix to use or which technology. Real-time PCR may offer excellent performance for this type of diagnosing. However such a method not only needs to be sensitive and specific but must be flexible and validated to handle different matrices, and fit together with a diversity of methods for sample extraction.

Objectives: The aim of this study was to analyse a wide range of matrices for VZV and HSV 1 and 2 using the affigene® VZV tracer and the affigene® HSV 1/2 tracer kits (Cepheid AB, Bromma, Sweden), respectively. The results from the commercial PCR assays were compared to in-house PCR assays.

Methods: DNA from 178 samples diagnosed for VZV and 352 samples diagnosed for HSV from 22 or 8 different matrices, respectively, were prepared using Qiagen blood mini kit (Qiagen, Hilden, Germany). Subsequently, the presence of VZV and HSV-1 and 2 was analysed using affigene® VZV tracer and affigene® HSV 1/2 tracer, respectively, on the Mx3000P instrument (Stratagene, La Jolla, USA). Fifty-two samples were also prepared using affigene® DNA extraction (Cepheid AB, Bromma, Sweden) according to the manufacturer's instructions and analysed using affigene® HSV 1/2 tracer. Prior to this study the samples had been analysed using Qiagen blood mini kit and an qualitative in-house PCR. Positive and negative agreement were calculated.

Results: Generally more positive samples were detected using affigene® assays in combination with the Qiagen blood mini kit preparation system compared to the in-house assays. In e.g. CSF six (of 40 samples) were detected positive for VZV using affigene® VZV tracer while only one using the in-house method. For HSV-1 detection the corresponding numbers in CSF were 5 compared to 3 of 40 for affigene® HSV 1/2 tracer and in-house assay, respectively. For HSV-2, 6 compared to none of 40 were detected with respective assay.

The positive agreement, for analysing VZV in all matrices was 85% and the negative agreement was 92%. For HSV-1 the corresponding figures were 97% and 89%, respectively, and for HSV-2 100% and 90%, respectively.

Conclusions: Affigene® VZV tracer and affigene® HSV 1/2 tracer are sensitive assays for detection of varicella zoster and herpes simplex viruses. A wide variety of sample matrices can be analysed using either Qiagen blood mini kit or affigene® DNA extraction as sample preparation method.

P994 Specific detection of hepatitis C virus in clinical samples using a novel simplification strategy

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Objective: We have shown previously that DNA simplification is a novel procedure that is able to specifically detect all high-risk strains of the Human Papilloma Virus in a single reaction. We wished to determine if it was possible to use a modified simplification strategy to detect RNA encoding viruses and used Hepatitis C virus (HCV) as a model organism.

Background: HCV is a major cause of chronic hepatitis, cirrhosis and liver cancer on a global scale. One of the most sensitive methods for the detection of HCV is reverse transcription PCR (RT-PCR), which in addition to improved sensitivity compared to serological methods, can also be used to monitor disease activity in response to anti-viral drugs. We aimed to produce a simplification-based RT-PCR assay for HCV RNA that was as sensitive and specific as current viral-load monitoring assays.

Methods: Treatment of DNA with sodium bisulfite results in the conversion of cytosine residues to uracil, which are subsequently amplified as thymine. Although such methods have been applied to the detection of DNA viruses such as HPV, the treatment of RNA with sodium bisulfite results in total degradation of the RNA and thus was of no clinical utility. We have developed a novel method for the simplification of RNA using sodium bisulfite, which eliminates RNA degradation and shows similar sensitivity and specificity to conventional approaches.

Results: Baseline sensitivity tests were conducted using serial dilutions of HCV (Acrometrix cat# 96–0203) diluted in normal human serum and purified using the Qiagen QiAmp UltraSens viral kit. Positive signals were generated from as little as 15 IU/mL of virus, indicating negligible RNA degradation. In addition, a number of commercially available panels were obtained including; BBI Diagnostics Genotype Panel (PHW202), Worldwide HCV panel (WWHV301), HCV RNA genotype performance panel (PHW202) and HCV RNA linearity panel (PHW804), all of which produced results indicating that the HGS simplification strategy performed as well as conventional RT-PCR. Furthermore, sequencing of amplicons derived from different genotypes demonstrated that the method could be used for genotyping individual

HCV strains. Finally the method was assessed on a blinded panel of clinical samples the results of which shall be discussed in more detail.
Conclusion: RNA simplification is a novel approach for the reliable detection and genotyping of RNA containing pathogens such as HCV or HIV.

P995 Development of immuno-polymerase chain reaction as a highly sensitive method for viral detection

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Objectives: To develop a more sensitive and rapid method for viral antigen detection, the study combined an immune response (antigen-antibody) and a polymerase chain reaction (PCR) to form a new method, immuno-PCR (IPCR). The goal is to enhance the detecting limit of the conventional enzyme-linked immunosorbent assay (ELISA).
Methods: A monoclonal antibody, mAbH3 or mAbN, which recognises the influenza A (H3N2) or a SARS-coronavirus recombinant antigen (GST-N), was covalently linked to a cytomegalovirus DNA fragment (394 bp) respectively to form a protein-DNA complex, mAbH3-DNA or mAbN-DNA. The complex was purified and used for the reporting second antibody. To compare the detection limits of the conventional ELISA and IPCR, first antibodies against influenza A or SARS-coronavirus antigens were fixed onto micro-well plates with serial diluted standard antigens (HA titer more than 1:160 or GST-N) as the targets. Conventional secondary antibodies and the antibody-DNA conjugates were used for reporting the antigen levels.
Results: By using a quantitative real-time PCR the calculated copy numbers of the mAbH3-DNA IPCR correlated with the levels of the target antigens with the detecting limit down to 1:1000 dilutions, while the detecting limit of the conventional ELISA was 1:10 under the same experimental conditions.
A similar experiment using the SARS-coronavirus antibody-DNA conjugates revealed that the detecting limit for GST-N was 2 ng/ml, while the detecting limit of the conventional ELISA was 30 ng/ml.
Conclusions: IPCR is more sensitive than conventional ELISA for viral antigen detection. It is promising to adopt IPCR to detect small amount of viral products.

P996 Evaluation of real-time PCR assay for early detection of Cytomegalovirus infection in solid-organ transplant recipients

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Objective: The aim of this study was to evaluate the advantages of Real-time PCR for early detection of CMV infection in solid-organ transplant recipients by comparison to four serology methods such as ELISA, Capture-ELISA, IFA, Western-blot.
Methods: The study included 90 solid-organ transplant recipients with clinical manifestations of recent CMV infection. Whole blood specimens and sera were collected from all patients. The whole blood specimens were processed with Real-time PCR (artus CMV LC PCR, QIAGEN) to detect CMV viral load, while the same assay was employed to detect EBV (LC EBV Quantification, Roche) and HSV/VZV (artus LC, QIAGEN) in 67 and 38 samples, respectively. All sera were tested for anti-Cytomegalovirus IgG/IgM antibodies by ELISA (AXSYM Abbott). IgM positive results were confirmed by Capture-ELISA (CE-Bouty), indirect immunofluorescene (IFA, Focus) and Western-blot (Wb-Immunogenetics) assays.
Results: CMV viral load was detected in 20 patients, while 7 of these patients had evidence of EBV co-infection(PCR+). EBV viral load was detected in 14 specimens, but no HSV or VZV DNA was detected. ELISA revealed an anti-CMV IgG positive rate of 90% in both PCR positive and negative patients. IgM antibodies were present in 15/20 (75%) CMV PCR positive patients and in 14/70 (20%) CMV PCR negative patients. The positive rates of the rest serology assays for the PCR(+) and ELISA IgM(+) patients were Capture Elisa (+) 84%, IFA (+) 73%, Western-blot (+) 88%, while the respective rates for the PCR(−) and ELISA IgM(+) patients were Capture Elisa (+) 50%, IFA (+) 35%, Western-blot (+) 43%. High viral load (>103 copies/ml) was detected in 10/15 patients with ELISA IgM positive results. Low viral load (<103 copies/ml) was detected in 5/20 patients with ELISA IgM negative results
Conclusion: The referring doctors are requested to provide more clinical informations. Double samples should be tested by all serology assays in order to access a safe diagnosis and eliminate the possibility of false negative and positive results. Evaluation of viral load by Real-time PCR seems to be more sensitive and specific for the early detection of CMV infection than the serology assays.

HIV

P997 Relationship between gastric secretion, bacterial and fungal colonisation and histological changes in gastric mucosa in HIV-infected patients

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Objectives: Abnormal secretion of acid gastric juice in the course of HIV infection may result in bacterial and fungal overgrowth in stomach. The aim of the study was to estimate gastric secretion in HIV-infected patients (pts) in relation to severity of immunodeficiency and route of HIV transmission as well as influence of changes in gastric secretion on bacterial and fungal colonisation of stomach and gastric mucosa histology.
Methods: 71 HIV-infected pts were studied, 35 with CD4 lymphocyte count <200/uL (group 1) and 36 with CD4 >200/uL (group 2). Biopsy samples were taken during endoscopy from corpus and antrum of stomach for histological analysis and gastric juice samples were taken for quantitative bacteriological and mycological analysis. Gastric secretion was evaluated in 37 pts belonging to group 1 (19pts) and group 2 (18pts) on the basis of BAO, MAO and PAO analysis. In this group, route if transmission in 21 pts was intravenous (drug addicts), whereas sexual in the rest of patients.
Results: However no statistically significant difference in BAO between the two groups was noted, statistically significant decrease in gastric secretion /p<0.05/ was found in group 1 in comparison to group 2 (MAO 11.8vs18.3; PAO 14.8vs22.7 mmol HCl/h). Such relationship appeared to be much more evident in pts infected via sexual route (MAO 7.4vs20; PAO 9.3vs25). Gastric juice culture in pts with MAO<10 mmol HCl/h in comparison to the rest of pts, revealed more frequent significant growth of both fungi (58%vs32%) and bacteria (33%vs8%). Pts with fungal colonisation $>10^4$ CFU/mL had lower BAO (1.8vs3.3), MAO (11.2vs17.5) and PAO (14.5vs21.5) in comparison to the rest of pts. Similarly, pts with bacterial colonisation $>10^4$CFU/mL had lower BAO (1.3vs3.0), MAO (8.8vs16.1) and PAO (11.2vs20.1). No statistically significant correlation was found between bacterial colonisation and histological changes in gastric mucosa except for higher prevalence of chronic active gastritis in corpus of stomach /p<0.05/. Fungal colonisation correlated with higher prevalence of chronic non-active gastritis in corpus of stomach /p<0.01/.
Conclusions: Significant decrease in gastric secretion was found in severely immunocompromised HIV-infected pts, provided they were not intravenously infected drug addicts, followed by more frequent bacterial colonisation which correlated with chronic active gastritis and fungal colonisation which correlated with chronic non-active gastritis in corpus of stomach.

P998 Differences in health resources use and associated cost between HIV immigrants and Spaniards

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Objective: The specific impact of immigration on health services utilisation and related cost have been hardly analysed. To compare resource utilisation patterns among HIV-infected immigrants and Spaniards and to estimate the direct healthcare costs of HIV/AIDS care in both groups of patients we designed this study.

Methods: All adult patients with HIV infection attended during the years 2003 – 2005 in a secondary teaching hospital. Immigrant was considered a patient non-born in Spain. We evaluated episodes of health resource consume: hospitalisation, any kind of out-patient visit, emergency room visit without hospitalisation, any kind of hospital-day care, out-patient surgery, and HAART dispensation. For the cost calculation, we used a unitary cost for DRG per admission, officially assigned standard costs for cost of individual out-patient and ER visits and real cost of Pharmacy. We compared all variables between immigrant and Spaniards.
Results: During the period of study 372 patients were attended, 12% immigrants. They generated 9662 episodes of health resources consume, 11.4% from immigrants. Number of hospital admissions per patient was higher in immigrants 1.3 (4.4) than Spaniards 0.9 (2.7), p = 0.03. There were no differences in case-mix 2.2 (1.4) and 2.1 (1) and unitary cost per admission (1511 and 1539€), number 9.2 (8.8) vs 9.8 (11.8) p = 0.85 and cost (46.9 (18.6) vs 45.3 (18.9) € p = 0.1) of out-patients visits per patient and ER visits (1.6 (1.7) vs 2.7 (4) p = 0.26 and ER cost per patient (94.1 vs 157.8 € p = 0.33) between immigrants and Spaniards respectively. The cost of HAART per patient was slightly higher in Spaniards 7351.8€ than immigrants 7153.9€, p = 0.01. The proportional distribution of health resources use was different: hospitalisation was more used in immigrants and ER visits in Spaniards. The global distribution of cost was similar between both groups, and 75% were attributed to HAART. Total cost per patient did not differ between both groups.
Conclusions: There were no major differences in the use of health resource and associated cost between immigrants and Spaniards, except for a higher use of hospitalisation in immigrant population. HAART dispensation is the main health resource used and principal reason for expense in both groups.

P999 HIV-associated pulmonary hypertension: to screen or not to screen?

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Objectives: Pulmonary hypertension (PH) is a well-known cardiovascular disease with a poor prognosis that occurs in HIV-infected patients with a frequency of 0.5% compared with the 0.02% in the general population. On April 2004 in the Latium Italian Region, with an estimate of 5000 HIV-infected subjects, a Registry of PH in HIV-infected subjects was instituted. HIV-infected patients diagnosed as having PH after undergoing a diagnostic algorithm for clinical suspicion of PH, were enrolled in the Registry. The availability of new therapeutic options for PH allowing a better prognosis led us to submit to an echocardiographic screening HIV-infected asymptomatic subjects followed-up at our outpatients clinic. Aim of this study was to estimate the frequency of PH in HIV infected patients without cardiopulmonary symptoms.
Methods: 510 consecutive HIV-infected outpatients with no symptoms or signs of cardiopulmonary disease underwent echocardiography to evaluate pulmonary systolic arterial pressure. Clinical characteristics of HIV-PH asymptomatic patients were then compared with those of symptomatic patients with HIV-PH enrolled in the Registry.
Results: Between April 2004 and November 2007, 23 out of 510 patients (4.5%) were found to have an elevated PSAP. Four out 23 (4/510, 0.8%) were classified as having HIV-PH according to Venice classification. Secondary causes of PH were identified in 16 patients. Three patients are still undergoing diagnostic procedure for classification of PH. Data on asymptomatic and symptomatic HIV-PH patients are shown in table 1.
Conclusions: HIV-PH could be more frequent than expected, and probably latent for many years. Since PH could complicate the course of HIV infection regardless the degree of immunosuppression, and represents a significant cause of morbidity and mortality, a systematic cardiopulmonary evaluation and follow up should be incorporated in the clinical management of HIV infected patients. An early diagnosis and a proper follow-up could ameliorate the prognosis of PH.

Table 1

	Symptomatic patients (N = 15)	Asymptomatic patients (N = 4)	p
Gender			
Males	9	4	0.2
Females	6	0	
Age[a] (years)	42±9	38±4	0.2
CD4+T-lymphocytes[a] (cells/mmc)	282±187	179±106	0.2
HIV-RNA[a] (copies/ml)	115,584±185,984	1202±825	0.03
PSAP[ba] (mmHg)	67±19	49±13	0.06
HAART[c]			
Yes	6	3	0.3
No	9	1	
Mortality			
Yes	4	0	0.3
No	11	4	

[a]Mean±SD; [b]PSAP: pulmonary systolic pressure; [c]HAART: highly active antiretroviral therapy.

P1000 Relationships between urinary IL-18, MMP-9 and BMP-7 concentration and renal lesion in HIV-1 infected patients

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Objectives: HIV infection of glomerular endothelial and mesangial cells can cause progressive renal lesion. IL-18 and MMP-9 which are overexpressed during an HIV-1 infection can stimulate proinflammatory response in the kidney, which can be counterbalanced by increased production of BMP-7.
Methods: A cross-sectional study was performed in 76 HIV (+) patients (57 male, 19 female, aged from 20 to 59 years, 58 on HAART) and 17 HIV (–) volunteers. Plasma HIV-1 RNA was evaluated using the Amplicor system. Urinary IL-18, MMP-9 and BMP-7 concentration was measured by ELISA (Ray Bio and R&D Systems respectively). Creatine clearance was calculated by using of Cockroft-Gault formula. In statistical analyses U Mann-Whitney and Spearman correlation tests were used.
Results: We observed the increase in urinary MMP-9 and the decrease in urinary IL-18 in HIV (+) patients in comparison to control group (6.09±1.39 v. 4.5±0.94 ng/ml, $p<0.05$ and 15.28±2.54 v. 42.66±11.02 pg/ml, $p<0.05$). There were no significant differences in urinary BMP-7 regarding to HIV (–) group (2.61±0.54 v. 3.16±0.68 pg/ml, p = 0.09). No significant correlation was found beetwen CDC classification and urinary IL-18, MMP-9 and BMP-7 concentration. The mean urinary MMP-9 concentration observed in patients undergoing HAART was significantly lower than in patients without ARV treatment (4.97±0.98 v. 10.18±4.76 ng/ml, $p<0.05$). HIV-1 RNA was positively correlated with urinary MMP-9 concentration in HIV (+) (r=0.29, $p<0.05$). Moreover urinary MMP-9 concentration was positively correlated with urinary BMP-7 concentration (r=0.34, p = 0.005) and negatively correlated with creatine clearance (r=-0.32, $p<0.05$) in studied population.
Conclusion: Increased urinary MMP-9 concentration is associated with plasma HIV viral load and could be a new biomarker of subclinical renal lesion. MMP-9 augment together with BMP-7 increase may be explained by compensatory protection mechanism against renal damage.

P1001 Heart rate variability in AIDS-patients co-infected with *T. cruzi*

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Background: Chagas disease caused by the protozoan *Trypanosoma cruzi* and transmitted by the triatomid bugs is highly prevalent in almost all Latin and Central American countries affecting between 11–13 million peolple. HIV infection is also high widespread in such countries. Cardiac autonomic impairment has been well characterised

in chronic chagasic cardiomyophaty as well as in HIV/AIDS related heart involvement.
Objectives: Evaluate the cardiac autonomic function by using the computarised analysis of heart rate variability in patients with the co-infection HIV/Chagas disease.
Methods: With the ethical approval, we studied 57 patients, gender- and age-matched, 19 (*T. cruzi*-HIV group), 19 (HIV group) and 19 (control group). A continuous ECG recording in DII lead was performed in supine rest, cold face and passive tilt tests and R-R intervals were acquired. The overall variability of R-R series was assessed in the time and frequency domains using autoregressive power spectral analyses.
Results: The groups were similar regarding to gender and age. The electrocardiographic's analysis of mean values of R-R intervals did not differ in the three studied groups (p= 0.814). In baseline, the HIV group showed a statistically significant increase of the low frequency area, compared with the co-infected group (p = 0.01), a decrease of normalised low frequency area, compared with co-infection group (p = 0.03), an increase of the high frequency area compared with co-infected (p = 0.03). The variance was significantly higher in the co-infected group compaired with the HIV-group (p = 0.03).
Conclusion: In baseline conditions, a shift of cardiac sympathovagal balance, suggestive of an impairment of sympathetic modulation, was detected in patients with the *T. cruzi*/HIV co-infection.

Table 1: R–R intervals, Variance and Spectral Components in Control, HIV and *T. cruzi*/HIV groups in baseline

Indices	Control Group (n = 19)	HIV (n = 19)	*T. cruzi*/HIV (n = 19)	P value
Mean RRi (ms)	856.72 (734–952.5)	854.05 (777.5–934.5)	824.52 (726.5–923.5)	NS
Variance (ms^2)	439.77 (489.3–889.45)	3346.36 (3622–717)*	4591.5 (249.5–1245.5)[#]	0.03
LF (Hz)	124.57 (0.09–0.11)	0.09 (0.08–0.11)	0.08 (0.06–0.1)	NS
LF power (ms^2)	152.13 (93.06–270.5)	826.26 (189.7–1104)*	980.7 (11.47–269.05)[#]	0.01
LF (n.u.)	27.76 (43.91–69.3)	42.05 (32.1–51.26)*	37.33 (15.93–58.07)*	0.03
HF (Hz)	58.08 (0.27–0.33)	0.29 (0.22–0.36)	0.31 (0.26–0.35)	NS
HF power (ms^2)	73.77 (62.34–154.9)	823.17 (106.1–824.05)*	1713.97 (22.97–386.4)[#]	0.03
HF (n.u.)	17.02 (21.3–37.95)	34.45 (20.76–49.52)	36.11 (20.89–48.73)	NS
LF/HF ratio	2.37 (1.14–3.24)	1.72 (0.73–2.37)	2.95 (0.24–3.45)	NS

A one-way ANOVA was used for statistical comparisons. RRi: R–R intervals; LF: Low frequency area; HF: High frequency area; LF n.u.: Low frequency area in normalised units; HF n.u.: High frequency area in normalised units.
*$p < 0.05$ in comparison to Control Group; [#]$p < 0.05$ in comparison to HIV group.

P1002 Bloodstream infections among human HIV-infected adult patients: epidemiology and risk factors for mortality

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Background: Bloodstream infections are a frequent complication found in HIV-infected patients and are usually associated with a poor prognosis.
Objectives: This study was undertaken to describe the epidemiology and sensitivity pattern of pathogens causing community-acquired (CA) and nosocomial (N) bloodstream infection (BSI) in adult HIV-infected patients along the fifteen last years, to establish risk factors for mortality and hence to give guidance in the choice of empirical antimicrobials.
Methods: The type of study was a retrospective analysis of BSI episodes prospectively collected through a blood culture surveillance program from January 1991 to December 2006. We used non-conditional logistic regression methods with death as dependent variable.
Results: 1,077 (6%) episodes of BSI occurred in HIV-infected patients out of 16,946 total episodes in the period of study. CA and N BSI were 634 (59%) and 443 (41%), respectively. *S. pneumoniae* and *S. aureus* were the most frequent pathogens (n = 279, 44%) in CA BSI. Coagulase negative staphylococci and *S. aureus* were the most frequent microorganisms isolated in N cases (n = 369, 38%). Cotrimoxazole resistance was common in CA and N BSI caused by Gram-negative bacilli (50% and 61%, respectively). However, resistance rates to ceftriaxone were low (3%). Crude mortality accounted for 140 cases (13%). The independent risk factors associated with mortality were: liver cirrhosis (OR: 2.90, 95% CI: 1.55–5.41, p= 0.001), corticosteroids treatment (OR: 3.51, 95% CI: 1.87–6.56, p< 0.001), neutropenia (OR: 2.21, 95% CI: 1.15–4.23, p= 0.02), inappropriate empirical therapy (OR: 2.44, 95% CI: 1.29–4.65, p= 0.006) and isolate of *C. albicans* (OR: 7.58, 95% CI: 1.61–35.65, p= 0.010).
Conclusions: BSI in adult HIV-infected patients was often caused by Gram-positive pathogens in both CA and N settings. An inappropriate empirical therapy and the presence of other immunosuppressive factors were independent risk factors for mortality. Ceftriaxone could be used as the initial empiric therapy for HIV-infected patients with suspected CA BSI.

P1003 Clinical performance of FXG:RESP (Asp +) assay for *Pneumocystis jirovecii* on respiratory specimens

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Objectives: *Pneumocystis* pneumonia continues to be a common infection in HIV patients and also occurs in other immunocompromised patients. Early diagnosis is known to improve outcome, and specifically, exclusion of the diagnosis, reduces the need for toxic empirical high dose cotrimoxazole therapy. Real-Time PCR offers the prospect of faster and highly sensitive detection of *P. jirovecii*. FXG:RESP (Asp +) [Myconostica, UK] is a test kit that detects both *P. jirovecii* and *Aspergillus* spp., utilising molecular beacons. In this report we focus on the clinical performance for *P. jirovecii*.
Methods: The FXG:RESP (Asp +) real-time PCR kit utilises the large subunit mtRNA gene as a target to detect *P. jirovecii* and the assay is highly sensitive, being able to detect 6 target copies. The assay appears to be specific for *Pneumocystis* spp., with the possible exception of *Fusarium solani*. It was tested blindly on 196 BAL samples, collected from 4 European hospitals. All results were compared to microscopy, usually Calcofluor or Gomori methanamine silver stains, performed shortly after the sample was collected, and in non-AIDS patients whether patient was treated for PCP. Most HIV/AIDS samples had been previously extracted and stored frozen as DNA; the remainder were extracted with the MycXtra™ fungal DNA extraction kit, having been stored frozen unprocessed.
Results: The kit contains an internal amplification control to detect potential inhibition of the PCR reaction and 6 (3%) of the clinical samples showed evidence of inhibition. These results were excluded from analysis, although 5 were positive by both microscopy and the FXG:RESP (Asp +) assay, and would be reported clinically. 42 samples were from HIV/AIDS patients and 148 from other patients, mostly with leukaemia. With respect to *Pneumocystis* detection, the FXG:RESP (Asp +) assay had good performance with a sensitivity of 97.4%, specificity of 92.9%, positive and negative predictive values of 90.4% and 98.1%. 8 samples had negative microscopy but very high FXG assay signals, suggesting that the microscopy was falsely negative, as reported in prior literature. These were reported as false positives
Conclusions: The clinical performance of the FXG:RESP (Asp +) assay for the diagnosis of *Pneumocystis* pneumonia is superb. Overall the speed of detection and sensitivity of the FXG:RESP (Asp +) assay should bring substantial clinical benefits. Prospective and supportive clinical trials are ongoing.

P1004 Effectiveness of vaccination against HBV infection in HIV-infected persons

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Objectives: The aim of the study was assessment of the efficacy of the standard vaccination against HBV among HIV infected patients and vaccination with additional doses in patients who did not develop the protective level of antibody after the initial vaccination. Influence of the stage of HIV infection was analysed.

Methods: 54 HIV infected individuals, 20 women (37%) and 34 men (63%), mean age 32 years were analysed. 32 patients (59.6%) were treated with antiretroviral drugs. Stage of HIV infection was assessed on the basis of data derived from medical records and immunological status at the moment of introduction of vaccination (CD4 cells count, viral load). Efficacy of vaccination was compared with control group (56 healthy volunteers). In both groups hepatitis B virus infection was excluded by serologic tests. HBvaxPro vaccine produced by Merck Sharp & Dohme Company, dose registered for adults was injected at month 0–1-6. Patients with anti-HBs < 10 IU/l have received booster doses of vaccine month intervals, no more then three.

Results: Protective level of antibodies was found in 52 (92.9%) persons from control group and 32 (63%) HIV infected individuals. Anti-HBs > 100 IU/l was twice more common in control group (80%) than in investigated group (46.3%) (p<0.001). Protective level of anti-HBs had 14.3% patients with CD4 below 200 cells/μl, none of them had anti-HBs > 100 IU/l. Patients with higher CD4 cell count had better response for vaccination (p = 0.015). Differences between patients with high and low viral load were not statistically significant (p = 0.015) but patients with viral load below 10 000 copies/ml had slightly better response then those with higher viral load. Efficacy of vaccination was also associated with the level of distraction of immunological system before introduction of HAART (patients with CD4 < 200 cells/μl or HIV-RNA > 50 000 copies/ml had worst immunological response for vaccination). After the fist additional dose of vaccine anti-HBs > 10 IU/l had 79.7% patients, 87.1% after the second dose and 90.7% after the third dose. Anti-HBs > 100 IU/l had subsequently 57.4%, 66.7%, 79.6% patients.

Conclusions: Effectiveness of the routine vaccination schedule was lower among HIV individuals in comparison with healthy volunteers. Influence of the progression of HIV infection on the response for vaccination was detected. Additional vaccine's doses have improved efficacy of immunisation which was comparable with general population.

P1005 Factors associated with presence of isolated anti-HBc antibody in HIV-infected persons in Taiwan

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Objectives: To determine factors associated with presence of isolated anti-HBc antibody (positive anti-HBc antibody with negative HBsAg and anti-HBs antibody) in HIV-infected persons in Taiwan where the prevalence of chronic hepatitis B virus (HBV) infection is 15–20% in the general population aged 25 years or greater and vertical transmission is the major transmission route for HBV.

Methods: Clinical characteristics of 403 HIV-infected persons aged 15 years or greater who had an isolated anti-HBc antibody pattern were compared with those of 943 HIV-infected persons who developed protective HBV immunity after HBV exposure (negative HBsAg; positive anti-HBs and anti-HBc antibody). HBV DNA was measured in 68 persons with isolated anti-HBc antibody who had available serum specimens. Among these 68 persons in whom 45 persons had available anti-HBs titers, the relationship between HBV DNA load and anti-HBs titer levels were analysed.

Results: In univariate analysis, factors associated with presence of isolated anti-HBc antibody included older age (41 vs. 37 years, p<0.001), sexual transmission route (66.6 vs. 60.1%, p = 0.033), lower median CD4 counts (203 vs. 295 cells/μgL, p<0.001), and high plasma HIV RNA load (4.65 vs. 4.44 log10 copies/mL, p = 0.008); HCV seropositivity and intravenous drug use were not independent associated factors. In multivariate analysis, independent associated factors were age (odds ratio, 1.022, 95% CI 1.011–1.034) and a CD4 count less than 100 cells/μgL (odds ratio, 1.605, 95% CI 1.236–2.083). Among the 68 persons with isolated anti-HBc antibody and HBV DNA measurement, 14.7% had detectable HBV DNA. Of the 45 persons with isolated anti-HBc antibody who had determinations of both anti-HBs antibody titers and HBV DNA load, 38 (84.4%) had titers between 1 and 10 mIU/mL. Only 13.2% of these 38 persons had detectable HBV DNA while 28.6% of the 7 persons with anti-HBs titer less than 1 mIU/mL had detectable HBV DNA load (p = 0.296).

Conclusion: We concluded that, different from what have been reported by investigators in the Western countries that chronic HCV infection is associated with isolated anti-HBc antibody pattern, age and CD4 counts lower than 100 cells/£gL were associated with presence of isolated anti-HBc antibody in HIV-infected persons in Taiwan, and compromised immunity with subsequently waning of anti-HBs titers might play a role.

P1006 Characteristics of patients with a short delay between the first HIV-positive screening test and the diagnosis of AIDS at Lyon University Hospitals, France from 1985 to 2007

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Objectives: A diagnosis of AIDS shortly after a detection of HIV antibodies suggests a long lasting course of disease without treatment. To explore a late access to care, we studied the factors associated with a short delay (Sh.D) between the first HIV positive screening test and AIDS.

Methods: Prospective anonymous cohort study of 2253 patients with AIDS followed since 1985 to 2007 with confirmed dates of the first HIV positive screening and AIDS (European AIDS definition) at Lyon University Hospitals. Population was stratified into 2 groups according to a delay > or ⩽3 months (M) between the first detection of HIV-1 antibodies and AIDS. Two periods before and after introducing HAART (1996) were analysed. Multiple logistic regression was used to identify the factors associated with a delay ⩽3 months (Sh. D) based on their odd-ratio (OR) and 95% confidence interval (95% CI).

Results: A total of 736 individuals (32.7%) were in the Sh.D. group. The multivariate analysis identified factors associated with a Sh.D: age (per 1 year increase) OR 0.95, [95% CI 0.94–0.97], male gender (OR 2.04, [95% CI 1.32–3.20]), heterosexual intercourse (OR 2.03 [CI 95%: 1.42–2.91]), injecting drug use (OR 0.30 [95% CI 0.15–0.58]) and other exposure of HIV infection (OR 2.31 [95% CI 1.49–3.56]) compared to homosexual intercourse, CD4 $<100/mm^3$ at AIDS (OR 1.69 [95% CI 1.24–2.29]), *Pneumocystis jirovecii* pneumonia as initial AIDS event (OR 1.84 [CI 95% 1.15–2.92]), oesophageal candidiasis (OR 0.37 [95% CI 0.21–0.64], lymphoma (OR 0.32 [95% CI 0.16–0.64] compared to Kaposi's sarcoma diagnosis, AIDS diagnosis after 1996 (OR 3.96 [95% CI 2.88.-5.44]) and two simultaneous opportunistic infections at AIDS (OR 1.55; 95% CI 1.08–2.21) compared to one.

Conclusion: Gender, route of infection, age at first positive screening, recent years of AIDS diagnosis and an advanced immunosuppression were independently associated with a Sh.D between HIV-1 infection detection and AIDS. These results provide opportunities to plane interventions to populations who are most likely to access lately to care after HIV infection. The reasons of a late access to care need to be explored urgently for individual and public health benefits

P1007 Seasonal variation of *Pneumocystis jirovecii* infection: analysis of underlying climatic factors

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Objectives: *Pneumocystis jirovecii* pneumonia (PCP) represents a serious opportunistic infection in immunocompromised patients. However,

the modes of transmission are still not completely elucidated. Seasonal changes of PCP incidence might be associated with climate changes. Therefore, we explored the relationship between PCP incidence and climatic factors.

Methods: Between April 1992 and December 2006, 576 microscopically confirmed cases of PCP were diagnosed at the Max von Pettenkofer-Institute serving the major hospitals in Munich, Germany, for laboratory diagnosis of *P. jirovecii*. The monthly incidence of PCP – both for the pre- and post-HAART area, respectively – were correlated with the three most important monthly weather data for Munich obtained from the German Weather Service., i.e. mean temperature (°C), height of precipitation as a measurement for rainfall activity (mm) and wind strength (Bft). Statistical methods used were bivariate graphical exploration and multiple linear regression modelling of the influence of weather parameters on the monthly incidence rates as dependent variable with adjustment for mean annual incidence and logarithmic transformation to achieve normal distribution.

Results: While there was a clear seasonal pattern in PCP incidence with two peaks in May and August, no significant correlation was found between PCP incidence and rainfall activity or wind strength. In contrast, PCP incidence was positively correlated with the mean monthly temperature. This correlation seems to be more pronounced in the post-HAART area than in the pre-HAART period.

Conclusion: To our knowledge, this is the first study using multiple linear regression statistics on monthly climatic data. In contrast to previous and more descriptive studies from Spain and the UK showing season-related PCP incidence patterns with maxima in the winter months – as it is known from other infectious respiratory diseases, we found an opposite incidence pattern with PCP incidence maxima in late spring and summer. Moreover, PCP incidence was positively correlated with the mean temperature. Possible explanations for this seasonal variety of PCP incidence will be discussed.

P1008 Anti-R7V antibodies prevalence in HIV-infected patients followed in the ambulatory care centre of a university hospital, Cotonou, Benin

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Background and Objectives: A cellular epitope (R7V) is acquired by HIV when the virus is released by budding. All known variants present this epitope at their external membrane. Induced anti-R7V antibodies were described in vitro to neutralise cell infection. Research also indicated an association between the slow or non progressor status of HIV infected patients and presence of anti-R7V Ab.

Objectives were to determine the prevalence of anti-R7V Ab in HIV patients followed in the Ambulatory Care Centre at the University Hospital of Cotonou, Benin, and to assess the relationship of the Ab with the clinical status of patients.

Methods: A cross-sectional study was carried out from May to November 2007. 131 consenting patients were enrolled (average age was 36 and sex ratio=0.5). Blood specimens were drawn at the inclusion visit; serum sample was collected, coded and stored at −20°C until anonymous testing. Samples were tested for anti-R7V by ELISA (Ivagen, Bernis, France). The statistical Pearson chi2 test was used to compare the presence of anti-R7V Ab and the variables, clinical status of the patients and treatment.

Results: For 114 patients among the 131, date of HIV diagnosis ranged from February 1999 to August 2007. Mode of contamination was unknown for all patients.

Anti-R7V Ab were found in 90.4% of patients HIV-diagnosed in 2006, in comparison of whose diagnosed in 2004 (43.4%), $p<0.01$. The prevalence of Ab was also highly significant ($p<0.01$) in the group of 60 asymptomatic patients (83.3%) versus the group of 71 symptomatic patients (59.1%). Less anti-R7V positive results were observed for patients under ARV treatment (53.2%) than for naive patients (85.5%), $p<0.001$. No difference was shown between immunocompromised patients (CD4 $\leqslant$200 cells/mm^3) and patients with no decline of CD4 ($\geqslant$500 cells/mm^3).

Conclusion: Prevalence study of the anti-R7V Ab in 131 HIV-infected patients showed: i) ARV treatment decreased the level of the Ab, as previously described; ii) it seems that there is no relationship with the immunocompromised status and decrease in the presence of Ab; iii) anti-R7V Ab are highly more frequent in HIV patients always asymptomatic, for whom the detection of this humoral marker could be recommended. A follow-up of the patients who tested positive for anti-R7V Ab will enable to determine whether the presence of Ab is predictive of a slower progression, or non progression toward AIDS.

P1009 Molecular epidemiology of HIV-1 in newly diagnosed patients in southern Spain

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Objective: To characterise the subtype diversity and determine the prevalence of resistance mutations in the reverse transcriptase (RT) and protease (PR) regions of pol in treatment-naive individuals at a tertiary medical centre in western Andalucia from 2004 to 2006.

Methods: We included 72 HIV-infected, treatment-naive patients receiving care in our Hospital. Patients were categorised as either recently infected or chronically infected. Patient age, sex, risk factor, coinfection status (hepatitis B and C), viral load, and CD4+ T cell counts were determined at the time of diagnosis. Plasma HIV RNA was quantified using the Amplicor HIV-1 Monitor Test 1.5. Viral sequences were amplified and the data were used to determine the subtype diversity, prevalence of resistance mutations in the RT and PR genes and for phylogenetic analysis.

Results: The subtype B virus was present in 88.9% of the individuals, 9.7% were infected with the circulating recombinant form CRF-02AG, and 1.4% with the subtype recombinant B/G. Of 17 newly infected patients 88.2% were infected with sybtype B, 5.9% were infected with CRF02-AG, and 5.9% with B/G. Of 55 chronically infected patients 89.1% were infected with subtype B and 10.9% were infected with CRF-02AG. Interestingly, 6 of the 20 individuals (30%) identifying heterosexual contact as their primary risk factor were infected with CRF02-AG, whereas only 1 of 43 individuals (2.3%) identifying homosexual contact as their primary risk factor were infected with CRF02-AG ($p<0.01$ chi-squared). One resistance-associated mutations (RAM) in either the RT or PR genes was present in 86.1% and 83.3% had RAM to one class of antiretroviral drug, whereas 2.9% contained RAM to two classes of antiretroviral drugs. No isolates had mutations associated with resistance to all three drug classes. No primary resistance mutations within the PR gene were observed. However, 86.1% contained at least one secondary mutation within the PR gene associated with PI resistance. M36I (41.7%) and L63P (48.6%), were the most common. Mutations in the RT region were less prevalent than in the PR region. Only two isolates had mutations in RT associated with resistance to NRTIs or NNRTIs. K103N mutation was present in 2.8%, while Y188C was present in 1.4%.

Conclusion: These results elucidate the subtype diversity present in this region and suggest that the transmission of highly resistant virus variants is not occurring at a high frequency in this population.

P1010 Prevalence of CCR5, CCR2 and SDF1 genotypes in general population and HIV seropositive individuals in Georgia

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Objectives: Host genetic factors might be one of the factors determining the rate and severity of HIV epidemic in particular country, and also is an important host factor for pathogenesis and natural history of HIV disease. Unlike CCR5 delta32 mutation, mutation CCR2–64I and SDF1–3A do not provide protection against HIV infection, but still play a role in the

HIV disease progression. The prevalence of HIV in Georgia remains very low (0.01% in general population, about 1% in IDUs). Taking into consideration high prevalence of HCV and HBV infections in general population (6% and 4% respectively) and even higher in high risk groups (in IDUs 68 and 59% respectively) in our country low prevalence of HIV is unexpected. Moreover, there is high rate of IDU and STDs in Georgia; and neighboring countries where high migration of Georgian population takes place already have wide scale HIV epidemic. The objectives of this study was to study the host genetic factors in the general population and HIV infected individuals in order to study the correlations between CCR5delta 32, CCR-64I and SDF1–3A genotypes and resistance to HIV infection and/or disease progression in our country.

Material and Methods: A cross-sectional study design was utilised to examine CCR5, CCR2 and SDF1 genetic variants. 139 persons representing a non pregnant general adult population and 120 HIV infected individuals were enrolled in the study. Mutations were detected by the polymerase chain reaction/restriction fragment length polymorphism method.

Results: Of 139 individuals from general population 2.8% were caring mutations in both CCR5 genes. However, overall allele frequency (6.37) was similar to the frequency among European population. No homozygous mutation was found among HIV infected group and the allele frequency was equal to 2.5. Allele frequency of CCR2 and SDF1 allele was 14.9 and 10.75 and 36.7 and 32 among two groups respectively.

Conclusions: The frequency of CCR2 and CCR5 mutations is comparable to the frequency among European population; however, the rate of homozygous mutated CCR5 delta gene is among the highest. Moreover, to our knowledge, the frequency of SDF1–3A allele frequency in Georgians is among the highest ever reported (the highest rate reported to date is 29.75 among Cretan Greeks). According to the results of our study the frequency of those gene mutations partially might be a reason for lower HIV prevalence in the county.

P1011 Seroprevalence of HIV in Russia (1987–2006)

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Objective: Currently the spread of HIV infection is acquiring an epidemic pattern in Russia. Thus, HIV incidence rates increased 24-fold in 2001, and the cumulative number of HIV cases reported in the country increased ten-fold compared to 1997–1998.The aim of the present study was to analyse the epidemiology of HIV infection in Western (WS) and Eastern (ES) Siberia over the period from 1987 through 2006.

Methods: Siberia consists of 14 administrative regions with total population of 32 115 697 citizens, which is about one fourth of total Russian population. The data from district STD clinics were obtained and registered in the departments of social statistics of the regional committees of statistics.

Results: HIV incidence rate in Siberia accounts for 17.0% of all HIV infected persons in Russian Federation. Additionally, the HIV incidence rate is higher than average in Russia by 13.1 percent. Trends in the incidence of HIV infection are shown in the Table (number of reported cases in Russia).

In 2002, HIV incidence rates in Irkutsk and Novosibirsk were 102.1 and 8.9 (per 100 000 inhabitants), respectively. More than 50% of all HIV cases were reported from intravenous drug users aged 19–29 years in 2004 (in comparison 80% in 2000). Males comprise 70% of newly diagnosed HIV cases. Sexual transmission was reported in 4.7% in 1999 and 54.3% in 2004. The sexual/intravenous routes ratio was 1/1.7 in 2004 (in comparison 1/21 in 1999).

	1987–1997	1998	1999	2000	2001	2002	2003	2004	2005	2006
Russia	7.02	10.95	26.41	79.88	173.00	226.00	264.99	303.27	331.55	385.30
Siberia	226	493	5.46	12.88	24.39	31.29	35.69	39.97	44.41	49.53
Novosibirsk (WS)	16	29	48	167	361	600	768	918	1.08	1.45
Irkutsk (ES)	22	47	3.29	7.99	11.56	14.06	15.65	17.38	19.43	21.50

Conclusion: At present HIV epidemic unevenly spreads in the Siberian region. There reservoirs of infection are already formed in some administrative territories such as Irkutsk (ES). As for Novosibirsk (WS), the process of formation is under way. Local intervention programmes are urgently needed to prevent the forthcoming HIV epidemic in Siberia.

P1012 Trend of prevalence of human immunodefiency virus type 1(HIV-1) non-B aubtypes in Galicia, northwestern Spain

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Objectives: HIV-1 non-B subtypes plays an important role in the HIV epidemic and its prevalence has increased throughout the years. Understanding the cause of this increase is important for public health strategies. The aim of this study was to observe the trend of prevalence of HIV-1 non B genotypes in Galicia (North West of Spain) between 2003 and 2007.

Methods: In a prospective study in Santiago de Compostela, from a total of 322 newly diagnosticated HIV patients, we defined subtypes by partial sequencing of the polimerase gene by means of automated sequencing (CEQ 2000 system, Beckman Coulter Inc) and phylogenetic analysis using the geno2pheno program (Max Planck Institut Informatic, http://www.geno2pheno.org).

Results: The subtype B was the most frequently isolated. The non-B subtypes proportion found between our patients were the following: non recombinant subtypes A, C, D, F and G (45.83%) and recombinant subtypes AE, AG, BF and BG (54.17%). Subtypes AG and D were the most prevalent and there were no diferences in relatives frequences of recombinant and non recombinant subtypes over the years. In newly diagnosticated HIV patients, the prevalence of non-B subtype viruses was: 3.17% in 2003, 2.59% in 2004, 1.49% in 2005, 12.72% in 2006 and 11.66% in 2007.

Conclusion: The present data suggest that a significant trend to higher prevalence in patients infected with non-B subtypes was observed and it became more intense for the two last years; however, HIV subtype B is still the most commonly found. On the other hand, comparing between the non-B subtypes we conclude that there are a similar proportion of recombinant and non recombinant non-B sybtypes.

P1013 Structural analysis of the RNA region preceding Gag start codon in HIV-1 type A variants circulating in Russia

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Objectives: There are three stem-loops in 5′-untranslated region of HIV-1 RNA playing important roles in viral replication: apical loop of DIS hairpin required for dimerisation of genomic RNA, SD hairpin essential for the generation of subgenomic mRNA and ø hairpin being the major determinant for specific packaging of virus-like particles. These hairpins are located in the region of approximately 100 nt length preceding Gag start codon.

The purpose of the study was to compare the structure of this region in different genetic HIV-1 subtype A variants characteristic for Russia among them the strains carrying the pol gene mutations A62V, V77I, A62V/V77I and no mutations (wild type).

Methods: We have studied 12 samples of PMBC from HIV-infected individuals belonging to four pol gene groups. Prior analysis of genes gag and env found HIV-1 subtype A in these patients. Sequences of the region preceding Gag start codon (650 bp) in nested PCR products were generated.

Results: We have found that the apical loop of DIS hairpin from almost all samples of HIV-1 subtype A in Russia had the same palindrome AG[GUGCAC]A typical for 84% samples of subtype A circulating in the world. The only one sample with A62V mutation characteristic for Russia had the palindrome structure GG[GUGCAC]A. One sample of wild type virus had the palindrome structure AG[GUGCAC]G. There

were no special features in the structure of SD and ø hairpins in Russian samples.
Conclusion: The substitution of A to G in one of three purine residues flanking the palindrome may play the essential role in the forming of structure "kissing-loop" of double genomic RNA of HIV-1. Our data also demonstrate that the structure of SD and ø hairpins is very conservative in HIV-1 subtype A. The structural peculiarities of the apical loop of DIS hairpins can be used in genotyping of virus subtype A.

P1014 Influence of p38 MAPK isoforms (alpha, beta, gamma and delta) in apoptosis induced by HIV-1 in CD4+ T lymphocyte-based cell lines

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Background: The CD4+ T cell reduction characteristic of HIV-1 infection is thought to result, in addition from infected T cell death, mainly from uninfected bystander T cell apoptosis. Nevertheless, the immunologic and virologic mechanisms leading to T cell death during HIV-1 infection are not yet fully understood.
Objective: In the present study we analyse the individual implication of the p38 mitogen-activated protein kinase (MAPK) isoforms (alpha, beta, gamma and delta) in apoptosis induced by HIV-1, taking into account that HIV-1 replication is known to be blocked by p38 inhibitors.
Methods: We used the SupT1 cell line where death induced by HIV-1 mainly happened by uninfected bystander cell apoptosis. A variety of SupT1-based cell lines were constructed constitutively expressing, under the control of cytomegalovirus promoter (PCMV), each dominant-negative p38 isoform and each wild-type p38 isoform as control. An EGFP marker gene, under the control of the HIV-1 promoter, was inserted in all of them. These cell lines were infected with HIV-1 and analysed by flow citometry.
Results: We found that survival in SupT1-based cell lines infected by HIV-1 was increased by dominant-negative p38alpha, p38gamma and p38delta isoforms, but not by the dominant-negative p38beta isoform. HIV-1 replication was delayed most by the dominant-negative p38delta isoform and to a lesser extent by dominant-negative p38alpha and p38gamma isoforms. Moreover, these three dominant-negative isoforms reduced apoptosis induced by HIV-1 in both infected and bystander cells.
Conclusions: These results suggest that, in SupT1 cell line, p38alpha, p38gamma and p38delta, but not p38beta, are implicated in HIV-1 replication and apoptosis induced by HIV-1 in infected and bystander cells.

P1015 HIV-1 induces apoptosis in primary osteoblasts and HOBIT cells through TNF-α activation

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Background: Several HIV-1 infected patients show bone loss and osteopenia/osteoporosis during the course of disease. The mechanisms underlying this degenerative process are largely unsettled and the relantionship between HIV-1 and osteoblasts/osteoclasts cross-talk regulation has not been yet investigated. The aim of our study is focused on analysis of biological effects of HIV-1 on osteoblasts and osteoblast-like HOBIT cells to determine the mechanisms involved in the bone loss in the course of HIV-1 infection.
Methods: Human hipbone osteoblasts of patients were obtained from commercial sources or isolated from HIV-1 negative patients enrolled after giving their informed consent. HIV-1 DNA was determined by PCR whereas RT-PCR was employed to determine viral RNA, cell membrane markers and TNF-a mRNA. Flow cytometry procedures were used for apoptosis and membrane markers analysis. TNF-α supernatant analysis was performed by commercial kit.
Results: Primary osteoblasts and HOBIT osteoblast-like cell line were challenged by specific HIV-1 X4 and R5 strains and proviral HIV-1 PCR analysis showed that these cells are not susceptible to infection. On the other hand, HIV-1, heat-inactivated HIV-1 and HIV-1 gp120 treatment induced a significant apoptotic process activation at 72–96 hours ($p<0.01$) that is tackled by soluble CD4 treatment suggesting an interaction between gp120 and cell membrane proteins. Hence, CD4 and co-receptors membrane protein and mRNA expression was assayed by flow cytometry and real time RT-PCR, respectively, in osteoblasts and HOBIT. Although the CD4 and CXR4 mRNA was costantly detectable in both the cell models, CD4 and CXR4 proteins are expressed at very low density in a low percentage of cells (4–6% and 4% respectively) whereas CCR5 is significantly more expressed. Finally, we analysed the mRNA and protein synthesis of TNF-α a pro-apoptotic factor in osteoblasts biology. HIV-1, heat-inactivated HIV-1 and HIV-1 gp120 treatment induced both the TNF-α mRNA and supernatant protein increase at 24–96 hours. Moreover, anti-TNF-α pretratment tackles the apoptosis induction suggesting a direct role of TNF-α in the HIV-1 related activation of apoptotic process.
Conclusions: These results indicate that HIV-1 triggers apoptosis in osteoblasts and HOBIT cells without infection but through the gp120 interaction with cell membrane suggesting a novel mechanism in the HIV-1 related impairment of the bone mass structure homeostasis

Antifungal treatment and prophylaxis

P1016 Caspofungin as first-line therapy of invasive aspergillosis in haematological patients: impact of baseline characteristics on response rate at end of treatment and survival

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Caspofungin (C) (70 mg day 1 and 50 mg daily thereafter) was evaluated as first-line therapy of probable/proven IA (first trial ever to strictly adhere to EORTC-MSG criteria) in 61 haematological pts receiving either standard chemotherapy or autologous HSCT (EORTC trial 65041). Confirmation of diagnosis and assessment of efficacy were performed by a Data Review Committee (DRC). The primary efficacy endpoint was the proportion of patients with complete and partial response at the end of C treatment (EOT), defined with standard response assessment criteria. Survival at day 84 was among the secondary endpoints.
In the Modified Intention to Treat analysis (including all patients with proven/probable IA receiving > 1 day of C treatment), 38 of 61 pts (62%) had acute leukaemia (mostly myeloid leukaemia) and 51 (85%) were neutropenic (<500 cells/mm^3) at study entry. Forty-six patients (75%) had uncontrolled cancer. Median age was 64 years (range 19–86). Fifty percent of patients had a Karnofsky score < 50. Median and mean duration of C treatment were 15 and 27 days, respectively (3–84). At EOT, 20 of 61 (33%) pts had a favourable outcome (95% CI 21%–46%), while 9 (15%) and 31 (51%) had stable and worsening disease, respectively. Survival at day 84 was 54% (33 of 61).
The response rate at EOT was not influenced by age, gender, type of underlying haematological disease, status of the underlying disease, certainty of diagnosis of IA, site of infection or presence of baseline neutropenia; however, the median Karnofsky performance score at start of C therapy was higher ($p=0.04$) in patients with a favourable outcome (median 70) compared to patients with an unfavourable outcome (median 50). Similarly, patients with stable disease had a higher performance score ($p=0.02$) compared to those without disease stabilisation.
Survival at day 84 was significantly influenced by the status of the haematological disease at start of C therapy (uncontrolled cancer 40%-controlled cancer 93%; $p<0.001$) and by the Karnofsky score at baseline (15% survival for score <50, 57% for a score 50–60 and 69% for score >60; $p=0.009$).
In conclusion, we identified Karnofsky performance score and status of the underlying haematological disease as significant baseline variables for response at EOT and/or survival at day 84. In future clinical studies

in IA, consideration should be given towards stratification of patients according to these baseline findings.

P1017 **Caspofungin use in daily clinical practice for treatment of invasive aspergillosis: results of a prospective observational registry**

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Objective: To prospectively assess real world experience with Caspofungin (C) administered as monotherapy or in combination therapy for initial or salvage treatment of proven or probable invasive aspergillosis (IA).

Methods: A prospective observational registry was developed to collect data from patients treated with C for a single episode of IA. Information was collected from Apr-06 to Sep-07. Clinical efficacy was determined by the local investigator as favorable (complete or partial) or unfavorable (stable disease or failure) at the end of C therapy (EOCT).

Results: One hundred and three consecutive patients with proven or probable IA (per EORTC/MSG criteria) were identified from 23 sites in 11 countries. Outcomes were assessed in 101/103 patients since 2 patients were not discharged from the hospital at study termination. The most frequent underlying conditions included malignancy (77%), allogeneic hematopoietic stem cell transplantation (HSCT, 20%), solid organ transplantation (SOT, 9%), autologous HSCT (8%), & HIV/AIDS (3%). Neutropenia at start of C was recorded in 57% cases. Eighty three patients (82%) were treated for probable IA. The majority (85%) of patients had pulmonary IA. In culture-proven cases, *A. fumigatus* was the most frequently isolated species followed by *A. flavus*. The majority of patients received C monotherapy (83%), primarily as salvage therapy (86%). Most salvage treatment (74.2%) occurred in patients refractory to prior antifungals. A favorable response at end of C treatment was seen in 56% (57/101) of patients with similar favorable outcomes for C used as combination therapy (56%; 9/16) or as monotherapy (57%; 48/85). Favorable response rates in significant subgroups were: malignancy 52% (41/79); allogeneic HSCT: 57% (12/21); neutropenia: 53% (35/66). Serious adverse events were reported in 4 cases (4%); only 2 patients (2%) discontinued treatment due to a drug related AE (respiratory failure & skin reaction). An overall survival rate of 73%(75/103) was observed at the EOCT.

Conclusions: In daily clinical practice, an overall favorable response rate of 56% was observed in patients with proven or probable IA treated with C monotherapy or combination therapy. Also, a favorable response rate of >50% was seen in high-risk patient sub groups, including those with malignancy, allogeneic HSCT, and neutropenia. These real-life findings are consistent with the findings observed in randomised clinical trials.

P1018 **The use of caspofungin in neutropenic patients with haematological malignancies and concomitant candidaemia**

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Objective: To evaluate the efficacy of caspofungin in neutropenic patients (pts) with hematological malignancies (HM) and candidaemia

Design: The study was conducted in 8 Hematology divisions; neutropenic HM pts with clinical evidence of infection and positive blood culture for *Candida* were enrolled.

Patients and Results: Between January 2005 and November 2007, 22 episodes of candidaemia were registered. All the 22 enrolled patients [M/F 16/6; median age 49 yo (range 19–71)] (11 AML, 4 ALL; 5 NHL, 2 MM) had received chemotherapy. Nine of them (41%) developed candidaemia during a HSCT procedure (6 allogeneic and 3 autologous). Before the infection onset and at start of caspofungin, all pts were neutropenic (ANC$<0.5\times10^9$/l) for a median average of 5 days (range 2–41). Other risk factors were: CVC (77%), steroids administration (41%), mucositis (73%), diabetes (13%), *Candida* colonisation (36%). Nine pts (41%) received systemic antifungal prophylaxis (5 fluconazole, 2 itraconazole, 2 voriconazole) for an average of 13 days (4–60). The most frequent symptoms were: fever (100%), dyspnoea (27%), jaundice (13%), diarrhoea (13%), shock (13%). Candidaemia diagnosis was made after a median time of 3 days (range 1–12) from the onset of fever; *Candida* species isolated were: *albicans* 9 pts (41%), *parapsilosis* 4 (18%), *krusei* 3 (14%), *tropicalis* 2 (9%), *dubliniensis* 1 (4.5%), *guilliermondii* 1 (4.5%), *famata* 1 (4.5%), *lusitaniae* 1 (4.5%). Caspofungin at standard dosage was administered as front-line treatment in 16 pts (73%); in the other 6 pts, previous antifungal therapy (3 L-AmB, 1 itraconazole, 1 fluconazole, 1 voriconazole) was switched to caspofungin after no more than 48 hours on treatment at candidaemia diagnosis. The median duration of caspofungin therapy was 13 days (range 6–26). No side effects (WHO >2) were registered. Antifungal treatment was efficacious with remission of fever and other symptoms and negative blood cultures in 15 pts (68%). At 30 days following candidaemia onset, 9 pts had died (41%), but the candidaemia was responsible for the mortality in only 4 pts (overall attributable mortality 18%).

Conclusions: Our data, reporting the largest series of neutropenic HM pts and concomitant candidaemia treated with caspofungin. This study confirms the efficacy and safety of this approach in a subset of very compromised pts similar to that reported in literature in non neutropenic pts.

P1019 **Efficacy and safety of caspofungin monotherapy in cancer patients with candidaemia**

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Objective: There is a paucity of data regarding the efficacy and safety of caspofungin (CAS) in cancer patients with candidaemia.

Methods: We retrospectively reviewed the medical records of all adult cancer patients with candidaemia (EORTC criteria) treated with CAS monotherapy for at least 3 consecutive days at MD Anderson Cancer Center (March 2001-February 2007). CAS was given intravenously at 50 mg /day after a 70 mg loading dose. Demographic characteristics, clinical and microbiological data, outcomes, minimal inhibitory concentrations (MIC) of *Candida* isolates (CLSI method), were recorded.

Results: We identified 65 such patients (20 or 31% had hematological malignancies). The clinical response and mycological response after 7 days of CAS was 80% (51 patients) and 78% respectively. The overall mortality was 5% for day 7, 12% for day 14, and 20% for day 30. The 30-day *Candida*-attributable mortality was 11%. Bivariate analysis showed that factors significantly associated with crude 30 day mortality were an APACHE II score >20, ICU admission, a positive culture of the tip of the removed central venous catheter, especially when CFU/ml was > 15, persistent fungaemia after initiation of treatment, and the presence of intercurrent bacterial infection. The 30 day attributable mortality in the 12 patients (18%) with fluconazole-resistant or susceptible-dose dependent *Candida* bloodstream isolates (MIC $\geqslant 8\,\mu$g/ml) was 25% vs. 8% in the 53 patients with fluconazole-susceptible *Candida* ($p < 0.05$). CAS was well tolerated.

Conclusion: Although selection of candidemic cancer patients who received CAS monotherapy might account for the observed favorable outcomes, in our experience CAS had comparable efficacy and safety to the ones described in candidemic patients without malignancy.

P1020 **Prospective, multicentre study of caspofungin for treatment of documented fungal infections in paediatric patients**

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Objective: A prospective open-label study was performed to obtain data on the efficacy & safety of caspofungin (CAS) in paediatric patients (ped pts) with invasive candidiasis (IC), oesophageal candidiasis (EC), or invasive aspergillosis (IA).

Methods: Ped pts ages 3 mos-17 yrs with proven IC, proven EC, or proven/probable IA (EORTC/MSG criteria) received CAS at 50 mg/m2 daily (70 mg/m2 on Day 1) as either primary or salvage monotreatment (max 70 mg/day). Favourable responses required complete resolution of clinical findings & microbiological (or radiographic/endoscopic) eradication (complete response) or significant improvement in these parameters (partial response). The primary efficacy assessment was at end of CAS treatment (EOT) and included pts with a confirmed diagnosis and receipt of atleast 1 dose of CAS.

Results: Of the 49 pts (33 at 6 mos-11 yrs & 16 at 12–17 yrs) enrolled, 48 had confirmed disease: 37 IC, 1 EC, & 10 IA. Most IC pts (35/37) had candidaemia & received CAS as primary treatment (31/37). Most pts with IA had pulmonary involvement (8/10, including 2 with >1 site of infection). All pts with IA were refractory & received CAS as salvage treatment. CAS was dosed for 2–87 days (median 10 & 38 days for IC & IA, respectively). Success at EOT was 30/37 (81%) in IC, 1/1 in EC, & 5/10 (50%) in IA. IC outcomes were generally similar across *Candida* spp. No pt developed a serious drug-related adverse event or discontinued CAS due to toxicity. All-cause mortality during treatment or the 4-week posttreatment follow up period was 10%.

Conclusions: In this study, CAS was effective & generally well tolerated in peds pts with IA or IC. Outcomes in peds pts are consistent with those reported in adults.

P1021 **Efficacy of caspofungin compared to itraconazole as secondary antifungal prophylaxis: a multinational case registry**

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Background: Patients surviving an invasive fungal infection (IFI) in need of further cytoreductive chemotherapy are at high risk for recurrent fungal infection. In the absence of randomised controlled trials on this issue, applied prophylactic regimens are diverse and rely primarily on the personal experience of the attending physician.

Methods: A multinational case registry of patients in need of secondary antifungal prophylaxis has been conducted from October 2001 onwards. Patients with leukaemia or high-grade lymphoma and a history of probable or proven IFI according to EORTC/MSG criteria were analysed if undergoing an additional episode of neutropenia and receiving prophylaxis with either itraconazole or caspofungin. Data collected comprise demographics, underlying disease, first episode of IFI, antifungal prophylaxis, incidence and outcome of breakthrough IFI, and survival.

Results: A total of 75 patients were evaluated, 47 receiving itraconazole and 28 receiving caspofungin. Age, gender, underlying malignancy, type and treatment of 1st IFI, and isolation measures were evenly distributed between both groups. Treatment of underlying disease more often was stem cell transplantation in the caspofungin group (21.3% vs 50%, P= 0.01). There were more patients with liver (0% and 25%, P = 0.001) or spleen (0% and 14%, P = 0.017) involvement of 1st IFI, with uncontrolled 1st IFI (0% and 10.7%, P = 0.049), or uncontrolled malignancy (8.5% and 32.1%, P = 0.012) in the caspofungin group. No significant difference for the occurrence of probable or proven breakthrough IFI (17.0% and 28.6%, P = 0.186) was observed. Treatment outcomes for recurrent IFI and underlying disease were similar between both groups. Mortality attributable to IFI (4.3% and 3.6%) and overall mortality (10.6% and 25%) were not different between both groups.

Conclusion: Both, itraconazole and caspofungin performed equally efficacious as secondary prophylaxis of IFI in high-risk patients. While there was a trend towards higher risk of recurrent IFI and death in the caspofungin group, these patients were at significantly higher risk probably influencing the choice of drug: They had a higher rate of uncontrolled fungal and underlying diseases and underwent stem cell transplantation more frequently. In spite of antifungal prophylaxis, risk of breakthrough IFI was high. Prospective controlled trials are warranted to define the best strategy of secondary antifungal prophylaxis.

P1022 **Efficacy and safety of micafungin for treatment of serious *Candida* infections in patients with or without malignant disease**

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Objectives: To evaluate the efficacy of micafungin for the treatment of invasive candidiasis/candidaemia in patients classified as having malignancy versus no malignancy at baseline.

Methods: Data (modified intent-to-treat population) were analysed from two phase III, multicentre, randomised trials conducted in patients with confirmed *Candida* infection at baseline. One trial, in adults and children, compared micafungin (adults: 100 mg/day; children: 2 mg/kg/day) with liposomal amphotericin B (L-AmB; 3 mg/kg/day); the other, in adults only, compared micafungin (100 mg/day and 150 mg/day) with caspofungin (50 mg/day; 70 mg loading dose). In both trials, the primary efficacy endpoint was treatment success at end of therapy, defined as both a positive clinical and positive mycological response.

	Treatment success at end of therapy, n/Nº (%)				
	MICA n = 244	L-AmB n = 245	MICA100 n = 191	MICA150 n = 199	CAS n = 188
No Malignancy	118/157 (75.2)	107/149 (71.8)	95/123 (77.2)	109/143 (76.2)	99/136 (72.8)
Malignancy	62/87 (71.3)	69/96 (71.9)	51/68 (75.0)	33/56 (58.9)	37/52 (71.2)
– without neutropenia	41/53 (77.4)	48/62 (77.4)	34/48 (70.8)	24/39 (61.5)	31/43 (72.1)
– with neutropenia	21/34 (61.8)	21/34 (61.8)	17/20 (85.0)	9/17 (52.9)	6/9 (66.7)

MICA: micafungin 100 mg/day in adults and 2 mg/kg/day in children; MICA 100: micafungin 100 mg/day; MICA 150: micafungin 150 mg/day; L-AmB: liposomal amphotericin B 3 mg/kg/day; CAS: caspofungin 50 mg/day with 70 mg loading dose.

Results: In the micafungin/L-AmB trial, there were 183 patients with malignancy (37% were neutropaenic) and 306 with no malignancy. In the micafungin/caspofungin trial, there were 176 patients with malignancy (26% were neutropaenic) and 402 with no malignancy. Key efficacy findings are shown in the table. In general, rates for treatment success with micafungin were similar in patients with and without malignancy and were similar to rates observed with L-AmB and caspofungin, although in one group (patients with malignancy, micafungin 150 mg/day, caspofungin comparator trial) treatment success was lower than in other groups (P = 0.15). Most patients with malignancy and neutropenia were successfully treated by all three drugs, although with the exception of patients receiving micafungin 100 mg in the caspofungin comparator trial, rates were lower (non-significantly) than in non-neutropaenic patients with malignancy. For all three drugs, the incidence of discontinuations due to treatment-related adverse events (AEs) was similar for patients with malignancy (⩽7.7%) versus no malignancy (⩽8.0%). In the L-AmB comparator trial, a smaller proportion of the total population discontinued therapy due to treatment-related AEs with micafungin (4.9%) versus L-AmB (7.9%). In the

caspofungin comparator trial, comparative values were 2.5%, 3.0%, and 3.6% for micafungin 100 mg, micafungin 150 mg, and caspofungin 50 mg, respectively.

Conclusion: Micafungin was effective and well tolerated in patients with or without malignancy. Micafungin is shown to be a valuable therapeutic option for the treatment of invasive candidiasis and candidaemia across a broad range of patients.

P1023 Pooled analysis of safety for micafungin

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Objectives: Micafungin (MICA) efficacy has been demonstrated in different patient populations [1–4]. To better understand the safety profile of MICA, a pooled analysis of clinical trial safety data was undertaken.

Methods: We analysed safety data collected from 15 clinical trials conducted worldwide. Adverse events (AEs) were coded using MedDRA 5.0. All patients (N=2653) received ⩾1 dose of intravenous MICA at a median daily dosage of 100 mg (range: 8.9–896 mg) for adults (aged 16–92 yrs) and 1.5 mg/kg (range: 0.4–4.7 mg/kg) for children (aged 0 wks-15 yrs), over a mean duration of 15 and 17 days, respectively.

Results: Mean subject age was 41.5 yrs (SD ± 18.7 yrs) including 226 (8.5%) children (<16 yrs) and 336 (12.7%) elderly patients (⩾65 yrs). Common underlying conditions were HIV (37.2%), haematopoietic stem cell or other transplantation (23.7%), and solid tumour or severe organ disorder (11.0%). Frequently reported MICA-related AEs (⩾2%) included phlebitis (2.8%), nausea (2.6%), vomiting (2.5%), hypokalaemia (2.2%), pyrexia (2.2%), and increases in alkaline phosphatase (2.6%), aspartate aminotransferase (2.5%) and alanine aminotransferase (2.0%). In comparative studies, the MICA safety profile was superior to liposomal amphotericin B, and similar to fluconazole and caspofungin (table).

Adverse event	Candidaemia/Invasive Candidiasis		Candidaemia/Invasive Candidiasis			Oesophageal candidiasis‡		Prophylaxis†	
	MICA 100 mg (n=316)	AmB 3 mg/kg (n=321)	MICA 100 mg (n=200)	MICA 150 mg (n=202)	CASPO 50 mg# (n=193)	MICA 150 mg (n=260)	FLU 200 mg (n=258)	MICA 50 mg (n=425)	FLU 400 mg (n=457)
Hypokalaemia	21 (6.6%)×	38 (11.8%)	4 (2.0%)	5 (2.5%)	3 (1.6%)	1 (0.4%)	1 (0.4%)	8 (1.9%)	8 (1.8%)
Pyrexia	23 (7.3%)×	39 (12.1%)	2 (1.0%)	0	1 (0.5%)	5 (1.9%)	1 (0.4%)	4 (0.9%)	5 (1.1%)
Rigours	2 (0.6%)×	19 (5.9%)	1 (0.5%)	2 (1.0%)	1 (0.5%)	6 (2.3%)	0	1 (0.2%)	5 (1.1%)
Creatinine increased	6 (1.9%)×	17 (5.3%)	0	1 (0.5%)	1 (0.5%)	0	0	1 (0.2%)	3 (0.7%)
Infusion-related event	52 (16.5%)×	87 (27.1%)	5 (2.5%)	0	5 (2.6%)	9 (3.5%)	8 (3.1%)	2 (0.5%)	4 (0.9%)

Treatment-related: assessed by investigator as having at least a possible relationship to study drug.
AmB: liposomal amphotericin B; CASPO: caspofungin; FLU: fluconazole; MICA: micafungin.
† Haematopoietic stem cell transplantation (HSCT) was the main inclusion criterion.
‡ Most oesophageal candidiasis (OEC) patients were HIV positive with AIDS.
× $P < 0.05$, Fisher's exact test.
After a 70 mg loading dose.

Conclusions: This large database with more than 2650 patients demonstrates that MICA has a favourable clinical safety profile.

Reference(s)

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P1024 Multivariate logistic regression modelling of variables associated with treatment outcomes from two large, prospective micafungin registrational trials

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Objectives: Two recent, large, prospective micafungin trials in patients (pts) with candidaemia and invasive candidiasis reported: (1) micafungin 100 and 150 mg were non-inferior to caspofungin (CID 2007;45:883; study 03–0–192), (2) micafungin was as effective as liposomal amphotericin B, with fewer adverse events (Lancet 2007;369:1519; study FG-463–21–08). Further analyses were undertaken to identify variables associated with overall treatment outcomes in adults.

Methods: Separate models were created for 03–0–192 (578 pts) and FG-463–21–08 (492 pts). Potential explanatory variables were age, gender, region, infection type, neutropenia status, baseline steroids, APACHE II score, catheter status, *Candida* spp., and baseline condition. Overall treatment success at the end of IV therapy (Rx) was defined as clinical and mycological success. Point estimates and 95% confidence intervals (CI) for the comparative odds for each variable were calculated.

Results: In 03–0–192, catheter removal, region, baseline APACHE II score, infection type, age group, and steroids had significant effects on treatment outcome (p = 0.0023, 0.0001, 0.001, 0.031, 0.041, 0.003, respectively). Odds ratio (OR) analyses demonstrated significantly less likelihoods to achieve treatment success for: (1) Pts without catheter removal (OR = 0.49, 95% CI = 0.31, 0.78), (2) Pts treated in India vs those in North America (OR = 0.14, 95% CI = 0.06, 0.29), (3) Pts with a baseline APACHE II score of >20 to ⩽30 or >30 vs ⩽20 (OR = 0.42 and 0.28, 95% CI = 0.24, 0.72 and 0.10, 0.77), (4) Pts with disseminated infection (OR = 0.36, 95% CI = 0.14, 0.91), (5) Pts ⩾70 yrs vs <50 (OR = 0.46, 95% CI = 0.25, 0.84), (6) Pts receiving steroids (OR = 0.50, 95% CI = 0.31, 0.79).

In FG-463–21–08, baseline APACHE II score and neutropenia status during Rx had significant effects on treatment success (p = 0.0001, 0.003). OR analyses demonstrated significantly less likelihoods to achieve success with: (1) A baseline APACHE II score of >20 to ⩽30 or above 30 vs ⩽20 (OR = 0.51 and 0.09, 95% CI = 0.30, 0.87 and 0.03, 0.27), (2) Neutropenia (OR = 0.32, 95% CI = 0.15, 0.68).

Conclusion: In both micafungin trials, pts with high APACHE II scores were less likely to have treatment success. Additionally, pts in 03–0–192 were less likely to achieve success if they did not have removal of catheters, were receiving Rx in India, had disseminated infection, were ⩾70 yrs, or were receiving steroids. Neutropenic pts in FG-463–21–08 were less likely to achieve treatment success.

P1025 Economic analysis of micafungin versus liposomal amphotericin B for treatment of candidaemia and invasive candidiasis in the UK

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Objectives: To investigate the economic impact of introducing micafungin (MICA) (Mycamine®) for the treatment of systemic candida infections (SCIs) (including invasive candidiasis and candidaemia), a health economic analysis was performed comparing MICA with liposomal amphotericin B (L-AMB) (AmBisome®).

Methods: The model was based on observations and time-points from a phase III, randomised, double-blind, clinical trial which compared MICA with L-AMB[1]. The model (originally developed for Germany) entails a period of 14–20 weeks starting from initiation of treatment and was analysed from a UK hospital perspective. Hospitalisation and primary medication costs were included in the current analysis. Unit costs of these resources were taken from appropriate UK costing sources. As the price for MICA was not available at the time of analysis, the price per recommended daily dose (RDD) of MICA (100 mg) was assumed to be equal to the price per RDD of caspofungin (50 mg). The model

endpoint was defined as the percentage of patients that achieved complete or partial clinical and mycological response after initial treatment, and were alive after the 12-week follow-up period. The model was analysed using two methods: a cohort simulation and a probabilistic simulation (i.e., Second-Order Monte Carlo [MC] simulation).
Results: The health economic analysis shows that with MICA 52.9% of patients were successfully treated and survived 12 weeks after treatment ends compared to 49.1% for L-AMB (see Table 1). In addition, MICA was, on average, less expensive than L-AMB costing £ 26,838 and £ 29,549 per patient, respectively

Table 1: Summary of results

Outcome	Treatment strategy	
	MICA	L-AMB
Average cost per patient		
Medication	£5,812	£6,580
Hospitalisation	£21,026	£22,969
Total	£26,838	£29,549
Effectiveness*	52.9%	49.1%
C/E	£50,755	£60,197
Incremental C/E ratio	L-AMB is dominated by MICA	

*% of patients that are succesfully treated and alive after 12 weeks.

Because the costs are lower and the effectiveness is higher for MICA (cost-effectiveness [C/E] ratio = £ 50,755) in comparison with L-AMB (C/E ratio = £ 60,197), MICA dominates L-AMB from a cost-effectiveness point-of-view. The results of the MC simulation and the sensitivity analyses showed that MICA remained the most cost-effective option throughout the analyses, although considerable variance in treatment-associated costs was observed for both treatment options.
Conclusions: The lower costs and higher effectiveness reported for MICA versus L-AMB in this analysis indicate MICA is the more cost-effective therapy in the treatment of SCIs when compared to L-AMB.

Reference(s)

[1] Kuse ER, Chetchotisakd P, da Cunha CA, et al. Micafungin versus liposomal amphotericin B for candidaemia and invasive candidosis: a phase III randomised double-blind trial. Lancet 2007;369(9572):1519–27

P1026 An evaluation of hospital length of stay in intensive care patients with invasive candidiasis treated with anidulafungin versus fluconazole

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Objectives: Invasive *Candida* infections in critically ill patients are associated with high attributable mortality and significant healthcare costs. The most significant cost results from an increased hospital length of stay (LOS) (Rentz et al). Although a number of antifungal drugs are available to treat these infections, few studies have compared the mean LOS associated with these agents. The objective of this study was to compare intensive care unit (ICU) and total hospital LOS in ICU patients receiving anidulafungin (ANID) versus fluconazole (FLU) for treatment of invasive candidiasis.
Methods: Data from patients confirmed to be in the ICU on day of entry into a phase 3, randomised double-blind study comparing intravenous (IV) ANID (200 mg Day 1, then 100 mg daily) and IV FLU (800 mg Day 1, then 400 mg daily) for treatment of invasive candidiasis (Reboli et al) were retrospectively analysed. ICU and total hospital LOS were determined for all eligible ICU patients and for those who survived to 2 weeks after end of treatment (EOT).
Results: Sixty-three of the 245 patients in the primary analysis population of the study were confirmed to be in the ICU at study entry (ANID, n = 35; FLU, n = 38). In this group, the mean ICU LOS was 23.4 ± 24.1 days for ANID versus 29.3 ± 26.2 days for FLU (difference, −5.9 days; 95% CI, −18.7 to 6.8), and the mean total hospital LOS was 32.6 ± 23.7 days for ANID versus 39.3 ± 29.9 days for FLU (difference, −6.6 days; 95% CI, −20.1 to 6.9). Fifty-four of the 63 ICU patients survived to 2 weeks after EOT (ANID, n = 32; FLU, n = 32). In this group, the mean ICU LOS was 24.8 ± 24.7 days for ANID but increased to 34.9 ± 27.0 days for FLU (difference, −10.1 days; 95% CI, −24.3 to 4.2), and the mean total hospital LOS was 34.9 ± 23.4 days for ANID compared with 47.3 ± 28.8 days for FLU (difference, −12.4 days; 95% CI, −26.7 to 1.9).
Conclusion: In ICU patients with invasive candidiasis, ANID treatment was associated with shorter ICU and total hospital LOS compared with FLU treatment, and these differences were more pronounced in patients who survived for 2 weeks after EOT. These numerical differences in LOS will likely have financial implications given the expense associated with prolonged hospitalisation and ICU usage.
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P1027 Cost-effectiveness of posaconazole versus standard azole treatment (fluconazole or itraconazole) in the prevention of invasive fungal infections among high-risk neutropenic patients in Spain

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Background: Posaconazole (POS) has demonstrated to be superior to standard azole therapy (SAT; fluconazole or itraconazole) in preventing both invasive fungal infections (IFIs) and reducing overall mortality among high-risk neutropenic patients (Cornely 2007).
Objectives: To evaluate the cost-effectiveness of POS compared with SAT for the prevention of IFIs among patients with acute myelogenous leukaemia (AML) or myelodysplastic syndromes (MDS) who are at high-risk of developing an IFI due to chemotherapy-induced neutropenia.
Methods: A decision-analytic model was used to predict the likelihood of IFI and death, using published data from the Cornely trial. Patients in the model were assumed to have received prophylaxis with POS or SAT (fluconazole, 81%; itraconazole, 19%). Clinical results were extrapolated to lifetime horizon by extending the model with 1-month Markov cycles including specific mortality risk for underlying diseases. Model outcomes include IFIs avoided, life-years saved, total costs, incremental cost per IFI avoided, and incremental cost-effectiveness ratio (ICER; incremental cost per life year saved) of POS versus SAT over a lifetime. The analysis was done from the perspective of the Spanish HealthCare System. Data on life expectancy were obtained from Spanish Statistics Institute. Information on medical procedures was obtained from the Cornely study and an expert committee. Costs for IFI treatment and drug/administration costs were taken from published literature (€2007 values). A deterministic and probabilistic sensitivity analyses (PAS) were performed.
Results: POS is associated with fewer IFIs (0.05 versus 0.11 p = 0.003), increased life years (2.52 versus 2.43), and (excluding costs of the underlying condition) significantly lower costs (€5,911 versus €7,475) per patient relative to SAT over a lifetime horizon. Results from the PSA indicate that there is an 87% probability that POS is a cost-saving strategy versus SAT and that the probability that the ICER ratio for POS versus SAT is below an estimated €30,000 per life year saved threshold is 98%.
Conclusion: Posaconazole has been shown to be more effective than standard azole therapy in preventing IFIs and reducing overall mortality in high-risk neutropenic patients. An incremental cost-effectiveness analysis done from the perspective of the Spanish HealthCare System, has indicated the dominance (lower costs and greater efficacy) of posaconazole versus fluconazole or itraconazole.

P1028 Cost-effectiveness of posaconazole vs. fluconazole in the prevention of invasive fungal infections among patients with graft-versus-host disease in Spain

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Background: Ullman et al. (NEJM 2007) had shown in a recent clinical trial that posaconazole (POS) is similar to fluconazole (FLU) in the prevention of invasive fungal infections (IFIs) among allogeneic hematopoietic stem-cell transplant (HSCT) patients with graft-versus-host disease (GVHD).
Objectives: To assess the cost-effectiveness of POS versus FLU in preventing IFI in patients with GVHD from the Spanish National Health System perspective.
Methods: A decision-analytic model was developed to assess the average per patient treatment costs, IFIs avoided, life-years gained, and incremental cost per life-year gained of each prophylactic treatment (€ 2007). The trial results were extrapolated to a lifetime horizon by extending the model with one-month Markov cycles including specific mortality risk for GVHD. Patients are assumed to receive POS or FLU; probability of IFI, IFI-related death and death from other causes were obtained from the published clinical trial. Long-term mortality and prophylaxis drug and IFI treatment costs were estimated from secondary sources. One-way and probabilistic sensitivity analyses were conducted.
Results: preliminary results shows that POS is associated with fewer IFIs (0.05 vs. 0.09), increased life years (8.01 vs. 7.78), and higher IFI-related costs (prophylaxis and IFI treatment) (€11.585 vs. €6.959) per patient relative to FLU over a lifetime horizon. Costs for treatment of IFIs comprised 82% of the total cost for FLU and 29% for POS. The incremental cost-effectiveness of POS versus FLU is estimated to be €20.246 per life-year saved. Results are most sensitive to changes in the cost of preventing an IFI and the efficacy of prophylaxis. A second-order probabilistic Monte Carlo sensitivity analysis was conducted to assess the effects of parameter uncertainty on the study findings, particularly as relates to treatment efficacy and the costs of preventing an IFI. Results indicate that there is a 70% probability that POS is cost-effective at a €30.000 per life year saved threshold, threshold commonly used in Spain for assessing the cost-effectiveness of new technologies.
Conclusion: Based on this analysis, we conclude that posaconazole is a cost-effective strategy in Spain for the prevention of IFIs among patients with GVHD.

P1029 Posaconazole versus standard azole therapy in the prophylaxis against invasive fungal infections among high-risk neutropenic patients in Canada: a cost-effectiveness analysis

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Objective: Posaconazole has been demonstrated to be significantly superior to standard azole therapy (i.e., fluconazole or itraconazole) in preventing invasive fungal infections (IFIs) ($P<0.001$) and in reducing overall mortality ($P=0.048$) among patients with haematological malignancy with prolonged neutropenia (Cornely et al. NEJM, 2007). The objective of this study was to assess the cost-effectiveness of posaconazole in this patient population from the perspective of the Canadian healthcare system.
Methods: A decision-analytical model based on trial results was developed. Patients in the model were assumed to receive prophylaxis with posaconazole or standard azole therapy (fluconazole, 81%; itraconazole, 19%). The probabilities of experiencing an IFI, IFI-related death, and death from other causes over 100 days of follow-up were estimated from the trial data. To extrapolate results beyond the trial period, the model was extended to a lifetime horizon using 1-month Markov cycles in which mortality rate is specific to the underlying disease as estimated from Statistics Canada and Surveillance, Epidemiology, and End Results (SEER) program data. Long-term mortality, drug costs, and IFI treatment costs were estimated using published literature. The model was used to estimate costs, IFIs avoided, life-years gained, and the incremental cost-effectiveness ratio (ICER) of posaconazole versus standard azole therapy (using 2007 Canadian dollars).
Results: Posaconazole is associated with fewer IFIs (0.05 vs. 0.11) ($P=0.003$), increased life-years (0.744 vs. 0.728), and (excluding costs of the underlying condition) slightly lower costs ($7,147 vs. $7,332) per patient relative to standard azole therapy over a lifetime horizon. A second-order probabilistic Monte Carlo sensitivity analysis was conducted to assess the effects of parameter uncertainty on the study findings, particularly as they relate to treatment efficacy and the costs of an IFI. Results indicate that there is a 53% probability that posaconazole is cost saving versus standard azole therapy and a 70% probability that the ICER for posaconazole is at or below the $50,000 per life-year saved threshold.
Conclusion: In addition to the proven efficacy, posaconazole appeared to be cost saving relative to standard azole therapy (i.e., fluconazole or itraconazole) in the prevention of IFIs among high-risk neutropenic patients.

P1030 Economic evaluation of posaconazole compared with other systemic fungal therapies in patients with refractory invasive aspergillosis in Canada

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Objective: To evaluate all-cause mortality and cost of treatment in patients with refractory invasive aspergillosis (rIA) treated with either posaconazole or other systemic anti-fungal (SAF) therapies.
Methods: All-cause mortality and cost of salvage therapy of posaconazole oral suspension (800 mg/day) and other SAF treatments were assessed using an open-label, multicentre, externally controlled study in patients with invasive aspergillosis who were refractory to or intolerant of conventional antifungal therapy. Data from external control cases were collected retrospectively to provide a comparative reference group. All patients had failed to improve or progress with prior antifungal therapy. Prior antifungal therapies for the majority of patients were liposomal amphotericin B, amphotericin B, or itraconazole. Cases of aspergillosis deemed evaluable by a blinded data review committee included 107 posaconazole recipients and 86 control subjects (modified intent-to-treat population). The populations were similar and balanced with regard to pre-specified demographic and disease variables. All-cause mortality were analysed using the survival technique. Cost analysis was censored at day 372. Economic evaluation were conducted using survival data and costs of pharmacotherapy (estimates are based on 2007 Canadian dollars).
Results: Significantly more patients who were treated with posaconazole responded to therapy compared with similar patients treated with other salvage therapies. Patients with rIA treated with posaconazole appeared to confer a highly significant survival benefit over the control cases. The cumulative rates of survival at 30 days and at the end of therapy were 74% and 38%, respectively; for control subjects, those survival rates were 49% and 22%, respectively. The Kaplan-Meier survival curve was significantly different ($P=0.0003$). In addition, posaconazole appears to be a "dominant" (i.e., cost saving) option for the treatment of refractory aspergillosis when compared to the active comparator receiving standard antifungal salvage therapies ($14,839 vs. $38,158). Sensitivity analyses demonstrated that the cost savings observed were maintained over a range of alternative values for costs and outcomes.
Conclusions: Treatment with posaconazole as compared with other systemic anti-fungal therapies provided a significant survival benefit in patients with rIA at lower cost of drug therapy.

P1031 **Pharmaco-economic analyis of posaconazole versus standard azole prophylaxis in high-risk neutropenic AML/MDS patients in Germany**

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Objectives: The objective of our analysis was to estimate the cost-effectiveness of antifungal prophylaxis with posaconazole vs. standard azole therapy (i.e., fluconazole or itraconazole) in patients with acute myelogenous leukaemia (AML) or myelodysplastic syndrome (MDS) and at high risk of developing an IFI due to chemotherapy-induced neutropenia. Posaconazole significantly prevents invasive fungal infections (IFIs) and reduces overall mortality vs. standard azole therapy in these patients (Cornely et al. NEJM, 2007).

Methods: A decision-analytic model was developed based on the results of the large multicentre trial, extrapolated to a lifetime horizon and analysed from a German perspective.

The probabilities of experiencing an IFI, IFI-related death, and death from other causes over 100 days of follow-up were estimated from the trial data. Long-term mortality data is based on the database of a large German university clinical centre, drug costs were estimated using published sources. IFI treatment costs were derived from a multicentre retrospective chart review. Due to a lack of published data on the impact of an IFI on quality of life of AML/MDS patients, a panel of 20 hematology-oncology experts estimated this impact based on the SF-6D questionnaire. The utilities derived from the analysis of the questionnaires served to estimate the quality-adjusted life years (QALY). The pharmacoeconomic model presented was used to estimate incremental cost per patient, incremental cost-effectiveness ratio (ICER) per life-years saved and incremental costs per quality-adjusted life years (QALY).

Results: Posaconazole is associated with fewer IFIs (0.05 vs. 0.11; p = 0.003) and slightly higher overall treatment costs (1,041 €) per patient relative to standard azole therapy over a lifetime horizon. The ICER per live-years saved was 10,235 €. Posaconazole prophylaxis resulted in 0.1 QALYs gained in comparison to prophylaxis with standard azoles. Results from a probabilistic sensitivity analysis indicate that there is an 81% probability that the cost per QALY gained with posaconazole is below 20,000 €, a commonly accepted threshold for cost-effectiveness.

Conclusion: Based on the clinical data and on the assumptions, our analysis demonstrated that posaconazole is likely to be cost-effective relative to standard azole therapy (i.e., fluconazole or itraconazole) in the prevention of IFIs among high-risk neutropenic patients.

P1032 **Retrospective, multicentre, pharmaco-epidemiological study of the administration of liposomal amphotericin B in patients admitted to intensive care units**

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Objective: To analyse the indications and posology of liposomal amphotericin B (L-AMB) in patients admitted to intensive care units (ICUs).

Methods: Retrospective, multicentre and observational study of patients admitted to ICUs and treated with L-AMB for any reason in 2006. Invasive fungal infections (IFIs) were classified as proven, probable or possible. Treatment indications were classified as first line or as rescue treatment.

Results: 179 patients were included and 30 ICUs participated. Mean APACHE II score was 20.9 (SD:7.9), mean length of ICU stay was 28.6 (SD: 24.0) days and intra-ICU mortality rate was 43.4%. Most common pathologies were medical pathology (45.3%) and surgery (34.1%) and 68.2% of the patients had severe sepsis or septic shock. Invasive fungal infections were proven, probable and possible in 43.6%, 15.6%, and 25.1% of cases, respectively and not classified in the remaining 15.7%. Aetiology: *Candida albicans* (38.0%), *Candida* non-*albicans* (15.1%), *Aspergillus* spp. (7.3%), two or more *Candida* spp. (12.3%). Mean duration of treatment was 15 days at a mean dose of 3.7 mg/kg/day. In 47.5% of the cases L-AMB was used as rescue treatment after fluconazole (40 cases) or caspofungin (30 cases) and as first line in the other 52.5% of the cases. Clinical response in evaluable patients (n = 354) was classified as satisfactory in 53.9% (n = 33) of the cases (72.6% in proven infection) and microbiological eradication in evaluable patients (n = 308) was achieved in 67.6% (n = 33) of the cases. Adverse events were detected on 51 patients but were classified as serious in only 4 (2.2%) patients. Severe renal failure requiring a change in the antifungal treatment was detected in only one case. There was no change in the mean creatinine value at the end of treatment in the patients treated with L-AMB, despite the fact that 109 (60.9%) were receiving nephrotoxic drugs concomitantly.

Conclusions: L-AMB was used predominantly in critically ill patients (haemodynamically unstable), both as first line and as rescue treatment above all for proven infections. In this group of patients satisfactory clinical response was very high. L-AMB was well tolerated, with little alteration of renal function even in patients taking concomitant nephrotoxic drugs.

P1033 **Influence of liposomal amphotericin B on the renal function of intensive care patients with elevated serum creatinine levels. A multicentre, retrospective, observational case collection study**

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Objective: To study the influence of liposomal amphotericin B (L-AMB) on the renal function of intensive care patients with elevated serum creatinine levels (SCL) at the start of L-AMB treatment.

Methods: This is a sub-analysis of a pharmacoepidemiological retrospective study of patients admitted to Spanish intensive care units (ICUs) during 2006 and treated for at least one day with L-AMB. For this sub-analysis, only patients with SCL >1.5 mg/dl at the start of treatment (baseline) were included. Primary endpoint was SCL at the end of L-AMB treatment. Secondary endpoints were: discontinuation of treatment due to nephrotoxicity, and differences among groups (SCL ⩽1.5 mg/dl at the end of treatment vs SCL >1.5 mg/dl at the end of treatment) by dose and duration of L-AMB treatment, along with differences in the percentage of nephrotoxic concomitant drugs administered.

Results: Of the 179 patients admitted to the pharmacoepidemiological study, 49 (27.4%) presented with SCL >1.5 mg/dl (median: 2.3 and range: 1.53–7.0) at baseline. Of these 49 patients 83.7% were haemodynamically unstable, APACHE II score was 23.3 (SD: 8.8), mean length of ICU stay was 28.6 (SD: 24.0) days and intra-ICU mortality rate was 63.3%. Median dose of L-AMB was 3.6 mg/kg/day (range 1–6) and median duration of treatment was 12 days (range 1–33). Concomitant nephrotoxic drugs were taken by 59.2% (n = 39) of patients. At the end of L-AMB treatment, 40.8% (n = 30) of patients presented with SCL ⩽1.5 mg/dl, and the remaining 29 patients maintained SCL >1.5 mg/dl. Of this latter group, only seven patients presented with higher SCL than at baseline, none of them 1.5 x basal line value. No patient discontinued treatment due to nephrotoxicity. Comparing both groups of patients, no differences among dose of L-AMB and duration of treatment were seen. In addition, no differences were observed between groups in the percentages of patients taking nephrotoxic concomitant drugs.

Conclusion: Although this is a small case collection study, L-AMB was safely used in haemodynamically unstable patients with deteriorated renal function. No differences concerning dose and duration of L-AMB treatment, and concomitant nephrotoxic drugs were seen between both groups. L-AMB is a treatment option for high risk patients with deteriorated renal function.

P1034 Effectiveness and safety of AmBisome® (liposomal amphotericin B) in combination with voriconazole in patients with invasive fungal infections. A multicentre retrospective study (RAVES study)

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Objectives: To study the efficacy and safety of the combination of AmBisome® and voriconazole in patients with invasive fungal infections (IFIs).
Methods: Retrospective, multicentre, data collection of patients treated with a combination of AmBisome® and voriconazole in Spanish hospitals. Data were included from patients treated for at least six days from October 2003 to November 2006. Infections were classified as proven, probable and possible according to the EORTC criteria. The primary endpoint was one month survival after drug study discontinuation.
Results: Thirteen patients were included in the study, eight were oncohematologic (five neutropenic) and five were intensive care patients. Four hematologic patients presented with a proven IFI (*Aspergillus flavus*, *Fusarium* spp., *Scedosporium* spp., and *Candida* spp.+unidentified mould), two with a probable IFI (*Fusarium oxysporum*, and Scesdosporium spp.) and two with a possible IFI (in one case cerebral toxoplasmosis was diagnosed in the necropsy study). Four intensive care patients presented with a proven infection (Candidaemia) and one patient showed a probable IFI (*Aspergillus flavus* and *Aspergillus fumigatus*). Doses of AmBisome® ranged from 2 to 5.5 mg/kg/day with a minimum treatment duration of six days and a maximum of 50 days. Two patients died within one month after study drug discontinuation: one patient after 48 hours and one after 30 days. Neither death was related to the fungal infection (one case cerebral toxoplasmosis and in the other one peritonitis). After discontinuation of study drug, a complete response was observed in nine patients, a partial response in three patients, and the patient with cerebral toxoplasmosis remained stable. Six patients experienced alterations in hepatic function, and in one of them voriconazole treatment was discontinued due to hepatotoxicity. No clinically relevant increases in serum creatinine levels (i.e. serum creatinine twice baseline level) were observed.
Conclusion: Although this is a small collection of cases, the efficacy results suggest no clinical antagonism of AmBisome® plus voriconazole in combination. Safety results suggest that this combination can be administered to high risk patients.

P1035 Safety of liposomal amphotericin as a once-weekly antifungal prophylactic regimen in very low birth-weight premature infants

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Objectives: *Candida* infections are a common cause of morbidity and mortality among very low birth weight (VLBW) infants. Several studies have shown that prophylaxis may decrease the incidence of candidiasis and improve survival. At a rate of 2.6/1000 patient days, a large number of infants will be exposed to drug to prevent one infection. The safety of a prophylactic drug then needs to be exhaustively evaluated. We studied the safety of liposomal amphotericin B (L-AmB) given once weekly at a 5 mg/kg dose as a prophylactic regimen to prevent *Candida* colonisation and invasive infection in VLBW < 6 weeks of age.
Methods: Premature newborns < 32 weeks gestation; <1500 g birth weight; < 7 days of life were eligible. Subjects were randomised to receive L-AmB 5 mg/kg once a week or placebo (dextrose in water). L-AmB trough level was obtained on day 7. Surveillance cultures were obtained at baseline, 72 hours later and then weekly from rectum, axillae and respiratory tract. Study drug was continued until no risk factors (antibiotics, central vascular access, hyperalimentation, endotracheal intubation) were present or 6 weeks of life which ever was earlier. Blood cultures were obtained as clinically indicated. Electrolytes, renal and liver function, as well as mortality and incidence of necrotising enterocolitis (NEC) and intraventricular haemorrhage (IVH) were monitored for safety.
Results: Twenty subjects were enrolled in to each study arm, demographic features and risk factors were evenly distributed. Steroids were used in 40% and 15% of L-AmB and placebo arms repectively. While on study, 5% on L-Amb and 15% on placebo arm developed colonisation; one subject on placebo developed invasive disease. One patient in each group died due to complications of prematurity. There was no significant difference in the incidence of IVH (grade III-IV), NEC or in the need for platelets, packed red blood cells and potasium supplementation. Renal and liver function changes were similar in both groups and of no clinical or statistical significance.
Conclusions: The safety of once weekly 5 mg/kg L-AmB in VLBW infants is supported by our trial and was comparable to placebo. Data from our pilot study suggests that a larger multi-centre trial is warranted

P1036 Comparison of antifungal therapy in intensive care unit and oncological patients

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Objectives. New antifungal agents have enlarged treatment options for invasive fungal infections. However, these drugs are more expensive than older agents such as fluconazole (FLU) or amphotericin B (AmB). The purpose of this study is to describe antifungal therapy (AFT) in ICU and oncological patients at a European tertiary care hospital.
Methods: Prospective observational, non-interventional study on 3 ICUs and 1 oncology ward (ONC) to assess AFT, reasons for treatment changes and clinical outcome in 50 consecutive patients (48% female, median age 53.5, range 23–74 yrs; 36 ICU, 14 ONC). Standard criteria for the diagnosis of invasive fungal infections (IFI) were used.
Results: Mean length of hospitalisation was 59 days, the mean length of ICU stay 25 days. Overall mortality was 30%, without statistical difference between ICU and ONC patients. The following risk factors for IFI were highly prevalent: neutropenia (58%), use of broad-spectrum antibiotics (94%), total parenteral nutrition (18%), intraabdominal surgery (24%). We evaluated a total of 58 AFT cycles (1582 treatment days), including 103 courses (data for initial courses in table). Mean duration of treatment was 8.4 days (amB) 15.4 (CAS), and 14.7 (VOR). Modification of initial AFT was necessary in 50% of cycles. Rates of premature change (PC) of AFT for individual agents were: AmB (73.3% overall, 53.3% due to adverse events (AE)/20% due to treatment failure), FLU (31.3, 12.5/18.8), VOR (70, 50/20), CAS (28.6, 0/28.6). The rate of PC due to AE was significantly higher with AmB and VOR than with FLU and CAS.

	ONC [38 cycles; (% of cycles)]	ICU [20 cycles; (% of cycles)]
Indication for AFT		
Increased colonisation index	1 (2.6)	5 (25)
Fever and neutropenia[a]	20 (52.6)	3 (5)
Possible IFI[a]	12 (31.6)	1 (5)
Probable/proven IFI[a]	2 (5.3)	8 (40)
Physician's decision	3 (7.9)	3 (15)
Agent used		
AmB[a]	15 (39.5)	0
FLU[a]	5 (13.2)	14 (70)
Itraconazole (ITR)	2	0
Voriconazole (VOR)	7 (18.4)	3 (15)
Caspofungin (CAS)	7 (18.4)	3 (15)
Other	2	0

[a]Significant difference between ONC and ICU patients.

Conclusion: AFT in oncology is still predominantly based on AmB, which has been replaced in ICU patients by newer agents (VOR, CAS). In oncology patients indication for AFT is rarely based on probable or proven IFI, while this diagnosis is more often established in ICU pts. Premature discontinuation of AFT is frequent with AmB and VOR and is usually caused by AE.

P1037 Antifungal use in intensive care units

M. Èižman and the ESAC Study Group

Objectives: To provide benchmarking data on antifungal use in all intensive care units in Slovenian general hospitals and teaching hospital to analyse total use and general use pattern.
Methods: Antimicrobial use data for 21 ICU from all Slovenian general hospitals and one University hospital were obtained from January to December 2006 as part of the national antimicrobial consumption surveillance. Use data of systemic antifungals at the level of the active substance were collected in accordance with the Anatomic Therapeutic Chemical (ATC) classification and Defined Daily Dose (DDD) measurement unit (WHO version 2006) and expressed as DDD per 100 bed-days.
Results: During the year 2006 11 333 (2.95% of total number) patients were hospitalised in ICUs. The average length of stay was 3.99 days. Mean systemic antifungal use was 22.82 (range 0.87–67.52) DDD/100 bed-days; in medical ICUs (n = 3) 15.31 (range 2.86–46.56), in surgical (n = 3) 30.87 (0.87–67.52) in interdisciplinary (n = 3) 14.10 (7.72–23.0) and in University medical ICU (n = 3) 15.50 and in surgical ICU 42.21 DDD/100 bed-days respectively. Fluconazol was the most frequently prescribed antifungal (mean 21.0 DDD/100 bed-days) followed by caspofungin (0.62), amfotericin B (0.60), voriconazol (0.51) and itraconazol (0.05).
Conclusion: Antifungal use was heterogeneous in Slovenian ICUs with the mean lying at 22.82 DDD/100 bed-days. Benchmarking data might provide a tool for assessing strategies that aim to optimise antifungal prescribing in ICUs.

P1038 Disinfecting power of multi-purpose soft contact lens disinfecting solutions on fungi colonising the conjunctiva and environmental air isolates

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Objectives: There has been increasing concern about fungal corneal infections since the outbreak of *Fusarium* infections in 2005 in soft contact lens wearers, which led to withdrawal of one multi-purpose disinfecting solution (MPS). Although FDA guidelines require MPS to reduce numbers of a type strain of *Fusarium solani* by one log dilution, their effectiveness on other strains is not routinely tested. Poor compliance with lens care routines and rubbing of the eyes can introduce fungi into the eye. This study investigated effectiveness of soft contact lens MPS against commensal and environmental fungal isolates.
Methods: The lower conjunctival surface and eyelashes of 30 subjects aged 20–24 were swabbed with a saline-moistened swab and cultured on potato dextrose agar with chloramphenicol (PDAc). Environmental fungi were obtained from ten cultures of PDAc exposed for 30 minutes in urban areas near to flower beds and lawns. Plates were incubated at 25°C for 7 days and fungal isolates sub-cultured, identified, and used as challenge strains against three soft lens MPS solutions using the stand-alone test required by FDA Guidelines. *F. solani* (ATCC 36031) was used as a control.
Results: Sixteen subjects were colonised with fungal organisms yielding a total of 23 isolates. Air sampling yielded a further 14 isolates. Isolates were mainly *Penicillium* (38%) and *Aspergillus* (30%) species, the latter being an ocular pathogen. Both MPS "A" and "B", which contain polyhexamethylene biguanide (PHMB), failed to achieve a one log reduction for any of the isolates though the reduction of *F. solani* was 1.3 log. MPS "C" which contains POLYQUAD was able to achieve a 1.0 or greater log reduction for all isolates.
Conclusions: MPS containing PHMB failed to successfully reduce viable fungal numbers by one log, although able to meet FDA Guidelines for the test strain of *F. solani*. Failure to inhibit such strains may increase the risk of ocular infection in contact lens wearers, as infections may be caused by other fungal pathogens, particularly *Aspergillus* spp. and poor compliance with lens care routines may lead to contamination with fungi. In the light of recent increase in microbial keratitis of fungal aetiology, changes to FDA guidelines to expand numbers of strains and species may be appropriate.

PK/PD: in vitro and animal studies

P1039 A daptomycin assay by high-performance liquid chromatography

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Objectives: Daptomycin (DAPT) is a new lipopeptide antibiotic with a unique mode of action. Its spectrum of activity includes most clinically relevant Gram-positive pathogens. With this drug there are few published pharmacokinetic data and these have been limited to healthy volunteers. With all new agents, it is important to be able to measure the serum concentrations, for an understanding of the drug's pharmacokinetics and for the management of patients with multiple organ failure. Our aim was to develop a high performance liquid chromatography (HPLC) method for therapeutic drug monitoring (TDM).
Methods: The column was a C-18, 5-micron (100 x 4.6 mm) with a guard column of the same material. The mobile phase was 0.2 M phosphate buffer, pH 5.5 and acetonitrile (70:30) and the flow rate, 1.5 mL/min. The samples were prepared by mixing with equal volumes of acetonitrile, left for 5 min and centrifuged (13,000g) for 5 min. Ten micro-litres of the supernatant was injected. DAPT was detected by UV absorbance (223 nm). The reproducibility, linearity, accuracy, precision, and the lowest limit of quantification (LLOQ) were investigated.
Results: The retention time (RT) was 6 min. The assay was reproducible: The % CV was 2.8 for a spiked serum containing 2.5 mg/L DAPT (n = 3). For a serum 5 mg/L DAPT sample, the intra-day assay precision %CV was < 4 (n = 32). The inter-day %CV was < 8. The assay was linear from 0.5 to 100 mg/L (R^2 = 0.9993). Serum recovery was > 93% when aqueous and serum samples 2.5, 20.0 and 100.0 mg/L were compared. The accuracy was excellent: the observed and the target concentrations (20, 40 & 80 mg/L) were closely correlated, with all the % errors <2.5. The LLOQ was 0.5 mg/L (n = 4, %CV was 5.2).
Conclusion: The HPLC method was reproducible, linear and accurate. It was straightforward and rapid with a RT of 6 min for DAPT and a total assay time of 10 min. It will be suitable for both a busy TDM laboratory and for the rapid processing of large batches of samples for research purposes.

P1040 Cidal activity of oral third generation cephalosporins against *Streptococcus pneumoniae* in relation to cefotaxime intrinsic activity

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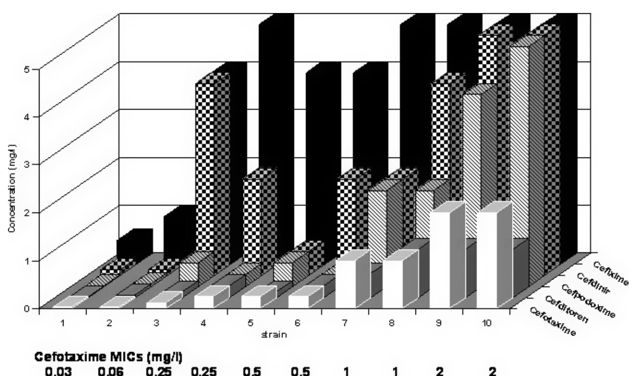
Objective: To explore killing kinetics within 12 h of oral versus parenteral third generation cephalosporins against ten *S. pneumoniae* strains exhibiting cefotaxime MICs from 0.03 to 2 mg/l.
Methods: Killing curves were performed following CLSI recommendations with double dilution concentrations ranging from 0.015 mg/l to 4 mg/l (concentrations achievable in serum after standard doses) and a final inoculum of 1–2 10^6 cfu/ml. Colony counts were performed at 0, 2, 4, 6, 8, 10, 12 and 24 h. The killing kinetic profile of each antibiotic was defined as the figure resulting when concentrations needed to obtain 90%, 99% or 99.9% reductions in initial inocula within 12 h (dosing interval for oral cephalosporins) are plotted against strains distributed by increasing cefotaxime MICs.

Results: The Figure shows the concentrations needed to obtain 90% reduction in initial inocula within 12 h for the 10 study strains (white columns: cefotaxime; gray columns: cefditoren; diagonal stripes: cefpodoxime; black squares: cefdinir; black columns: cefixime).



Conclusion: The similarity of the killing kinetic profiles of cefditoren and cefotaxime may be important when choosing an oral third generation cephalosporin as initial or sequential therapy in areas with high penicillin resistance prevalence

P1041 Critical evaluations of generic piperacillin/tazobactam compared to the branded product: a worldwide sampling of 26 intravenous formulations

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Objective: To evaluate generic piperacillin/tazobactam (P/T) intravenous product activity by diffusion and MIC assays compared to branded product (Zosyn® or Tazocin®; Wyeth). P/T is a widely used penicillin/β-lactamase inhibitor combination (8:1 ratio). Recently, generic formulations have been introduced into various global markets said to possess bioequivalence and the original brand was reformulated using proprietary methods to enhance quality.

Methods: We studied "non-branded" generic lots for antimicrobial potency against 4 assay organisms (triplicate testing; P/T MICs, 1–4 mg/L), and directly compared to current branded agent. Generic P/T products (26 samples from 23 lots) were from Greece (3 lots), India (5), Philippines (10), Portugal (1), Taiwan (2), China (2), Jordan (1) and Spain (2). CLSI susceptibility testing applied reconstituted product sample vial contents to prepare panels having 20 dilution steps between 0.5–8 mg/L. Each strain was tested and the lowest reproducible MIC value was used for calculating lot potency compared to control (Zosyn® lot B75011).

Results: All tests were performed on the same day from fresh stock solutions. 4 manufacturers (Astral, Meditrina, YSS and Zurentus) had 2 or more lots. The branded formulation consistently produced the lowest MIC results (range, 1.0–3.5 mg/L) and generic products had MIC results that were consistently elevated, indicating reduced activity varying from –3 to –35% (ave. –16%; only Orchid Piptamate was equal). Largest differences were observed with Zopercin (151018; –21%), Tazidron (07077; –27%) and Vigocid (8001C; –35%), and the tazobactam effect also appeared diminished. A second sampling of 3 lots (PIPTAZ AUPM-601 and AUPI-601; Vigocid 8002C) exhibited consistently decreased activity that varied from –3 to –20%, demonstrating reproducibility of the MIC-based assay.

Conclusions: In vitro activities of various formulations of intravenous P/T products can vary significantly as assessed by this incremental MIC assay system when applying product vial content to reference dilution testing. Per-label activity was noted for the branded formulation and all but one generic lot (among 26 sampled from 15 manufacturers) had decreased potencies. Hospital formularies should be cautious when applying generic products without well-documented equivalence by chemical parameters, biologic tests related to in vivo bioavailability or clinical outcomes and direct in vitro potency assays.

Product (no. lots)	Assay organism MIC (mg/L)			% variation
	E. coli (2)	*P. aeruginosa*	*S. aureus*	
Zosyn control (1)	1.75–3.5	2.0	1.25	NA
STADA (2)	2.5–5.0	2.5–3.5	1.25–1.5	–15 to –23
Tazorex (1)	2.5–5.0	2.5	1.25	–23
Tazotum (2)	2.5–5.0	3.0	1.25–1.5	–11 to –16
Tazidron (2)	1.25–5.0	2.5	1.25	–13 to –27
Piptamate (1)	1.75–3.5	2.0	1.25	0

P1042 In vitro activity of tigecycline against *Stenotrophomonas maltophilia* tested by time-kill studies

B. Aracil, Y. Gil, J.L. Gómez-Garcés (Móstoles, ES)

Introduction: The pharmacodynamic activity of tigecycline for *Stenotrophomonas maltophilia* are not defined.

Methods: Antimicrobial susceptibility testing of 80 clinical isolates from 1996 to 2006 was performed by agar dilution. Tigecycline concentrations were serially increased in time-kill studies with representative strains of different susceptibility, MIC of 0.5 mg/liter, and MIC of 8 mg/liter. The in vitro susceptibility of the strains was tested by time-kill studies in duplicate. From an initial inocule gives of 0.5 105 ufc/ml serially counting were done at 0, 2, 4, 6, 24, and 48 h.

Results: Eighty isolates were tested and were found to have the following antimicrobial susceptibility profiles: tigecycline, MIC50 of 2 mg/liter and MIC90 of 4 mg/liter; ciprofloxacin, MIC50 of 1 mg/liter and MIC90 of 8 mg/liter; colistin, MIC50 of 16 mg/liter and MIC90 of 32 mg/liter; gentamycin, MIC50 of 16 mg/liter and MIC90 of 32 mg/liter; ceftazidime MIC50 of 32 mg/liter and MIC90 of 32 mg/liter; piperacillin-tazobactam MIC50 of 128 mg/liter and MIC90 of 128 mg/liter, and imipenem, MIC50 of 32 mg/liter and MIC90 of 32 mg/liter. According to FDA breakpoints 81.2% of the isolates were susceptible to tigecycline, 12% of the isolates were considered intermediate and 3 isolates were resistant. A reduction of 0, 0, 1, 2.2, 3, and 3 log10 was produced at 0, 2, 4, 6, 24, and 48 h., respectively.

Conclusion: Concentration escalation studies with tigecycline revealed a maximal killing effect near the MIC with no additional extent or rate of killing at concentrations 2× to 4× the MIC for tigecycline with independence of the categorisation were susceptible or resistant.

P1043 In vitro activity of tigecycline against *Acinetobacter baumannii* tested by time-kill studies

B. Aracil, Y. Gil, J.L. Gómez-Garcés (Móstoles, ES)

Introduction: Tygecicline is a new expanded broad spectrum antibiotic against *Acinetobacter baumannii*, its pharmacodynamic activity is not well defined yet.

Methods: Antimicrobial susceptibility testing of 85 clinical isolates from 1996 to 2006 was performed by agar dilution. Tigecycline concentrations were serially increased in time-kill studies with representative strains of different susceptibility: MICs of 0.5 mg/l, 1 mg/l., 2 mg/l, and 4 mg/l. The in vitro susceptibility of the strains was tested by time-kill studies in duplicate. From an initial inocule gives of 0.5 105 ufc/ml serially counting were done at 0, 2, 4, 6, 24, and 48 h.

Results: Eighty-five isolates were tested and were found to have the following antimicrobial susceptibility profiles: tigecycline, MIC50 of 0.25 mg/liter and MIC90 of 2 mg/liter; ciprofloxacin, MIC50 of ⩽0.25 mg/liter and MIC90 of 8 mg/liter; colistin, MIC50 of 1 mg/liter and MIC90 of 2 mg/liter; gentamycin, MIC50 of ⩽1 mg/liter and MIC90 of 32 mg/liter; ceftazidime MIC50 of 8 mg/liter and MIC90 of 32 mg/liter; piperacillin-tazobactam MIC50 of 32 mg/liter and MIC90 of 128 mg/liter, and imipenem, MIC50 of ⩽1 mg/liter and MIC90 of 32 mg/liter. According to FDA breakpoints 96.5% of the isolates were

susceptible to tigecycline. A reduction of 0, 0, 0, 1.2, 3.5, and 3.5 log10 was produced at 0, 2, 4, 6, 24, and 48 h., respectively.
Conclusion: Concentration escalation studies with tigecycline revealed a maximal killing effect near the MIC, with no additional extent or rate of killing at concentrations 2× to 4× the MIC for tigecycline for the susceptible and intermediate strains.

P1044 Bactericidal activity of simulated serum concentrations of daptomycin vs. vancomycin against vancomycin-susceptible and resistant *Enterococcus faecium*: a pharmacodynamic model

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Objective: To assess the influence of protein binding (36.9% for vancomycin – VAN – and 91.7% for daptomycin – DAP –) and the decreased susceptibility to VAN in *E. faecium* on the serum bactericidal activity of VAN and DAP over the dosing interval.
Methods: Serum concentrations after iv 6 mg/kg od DAP vs. 1000 mg bid VAN were simulated in an in vitro computerised one-compartment dynamic model using Mueller Hinton broth supplemented with 4g/dl human albumin and 100 mg/l calcium. Initial inocula: 7.0 log10 cfu/ml. Antibiotic concentrations and colony counts were determined (in triplicate) over 24 h. Pharmacokinetic parameters were determined and AUCall was calculated by the linear-log trapezoidal rule. *E. faecium* strains (S) were chosen based on VAN MIC/MBC (mg/l): S1(1/32); S2 (2/64) and S3 (128/>128), with DAP MIC/MBC values of 2/16, 2/8 and 4/16 mg/l, respectively.
Results: Cmax (mg/l), t1/2 (h) and AUCs (mg/l x h) were: 44.5 ± 2.6, 6.0 ± 0.5, and 286.5 ± 13.1 for VAN and 85.7 ± 5.8, 8.1 ± 0.2, and 852.7 ± 15.8 for DAP, respectively. Initial inocula ranged from 7.0 to 7.3 log10 cfu/ml. Mean ± SD colony counts (log10 cfu/ml) are shown in the table.

		4 h	6 h	12 h	24 h
VAN	S1	7.0±0.1	6.8±0.2	6.3±0.3	5.6±0.2
	S2	6.5±0.1	6.5±0.1	6.4±0.4	7.3±0.3
	S3	7.7±0.0	8.2±0.2	8.9±0.2	8.9±0.2
DAP	S1	4.7±0.6	4.0±0.6	2.6±0.4	3.8±0.7
	S2	5.1±0.1	4.9±0.1	4.5±0.1	4.0±0.6
	S3	4.7±0.0	4.0±0.2	3.1±0.5	3.6±0.3

Conclusions: DAP exhibited bactericidal activity (>=3 log10 reduction) at 24 h regardless VAN susceptibility, MBC values or presence of albumin physiological concentrations. VAN was not bactericidal (<1.5 log10 reduction) even against VAN-susceptible strains.

P1045 In vivo fluoroquinolone concentrations and in vitro selection of resistance in *Streptococcus pneumoniae*

E. De Vecchi, L. Nicola, S. Zanini, L. Drago (Milan, IT)

Objective: To compare the ability to select for resistance in *S. pneumoniae* of levofloxacin (LVX), ciprofloxacin (CIP), prulifloxacin (PRU) and moxifloxacin (MXF), which are commonly prescribed for treatment of community acquired infections in Italy.
Methods: Fluoroquinolones susceptible *S. pneumoniae* (n = 30) isolated from lower respiratory tract infections were used. Frequency of spontaneous single-step mutation allowing for bacterial growth in presence of plasma and epithelial lining fluid (ELF) peak and trough concentrations were calculated. Multistep selection of resistance was evaluated by performing 10 serial subcultures on antibiotic-containing agar plates, in which a linear gradient ranging from the peak to the trough concentrations of each drug was obtained, followed by 10 subcultures on antibiotic-free agar. LVX was tested at concentrations obtained with 500 mg and 750 mg dose. MICs were determined after 1, 5, 10 passages on antibiotic-gradient plates and after 10 subcultures on antibiotic free agar. Resistant strains selected after multistep selection were characterised for DNA mutations by sequencing gyrA, gyrB, parC and parE genes.
Results: LVX and MXF showed the lowest frequencies of mutations (median $<11^{-11}$) at plasma peak and at all ELF concentrations, while medians ranging from 10^-8 to 10^-6 were observed for CIP and PRU. In multi-step selection of resistance, CIP and PRU selected for the highest number of resistant strains (19 and 31, respectively. LVX selected for 2 resistant strains at plasma concentrations. No selection of resistance was observed for LVX ELF concentrations and for plasma and ELF MXF concentrations. Mutations in parC, parE and gyrA genes were found in CIP and PRU resistant strains, while only parC mutations were found for LVX.
Conclusions: LVX and MXF, which are known to possess the highest activity against *S. pneumoniae* among the tested fluoroquinolones, are also characterised by a lower capacity than CIP and PRU to in vitro select for resistance when tested at concentrations occurring in vivo.

P1046 In vitro selection of resistance by in vivo concentrations of fluoroquinolones in *Pseudomonas aeruginosa* and *Staphylococcus aureus*

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Objective: To compare the ability to select for resistance in *Staphylococcus aureus* and *Pseudomonas aeruginosa* of levofloxacin (LVX), ciprofloxacin (CIP), and prulifloxacin (PRU).
Method: Twenty strains of *S. aureus* and of *P. aeruginosa* susceptible to fluoroquinolones, isolated from lower respiratory tract, mainly of nosocomial origin, were considered. Frequency of spontaneous single-step mutation allowing for bacterial growth in presence of plasma and epithelial lining fluid (ELF) peak and trough concentrations were calculated. Multistep selection of resistance was evaluated by performing 10 serial subcultures on agar plates containing a linear gradient ranging from the peak to the trough concentration of each drug, followed by 10 subcultures on antibiotic-free agar. LVX was tested by considering concentrations obtained after 500 mg and 750 mg administration. MICs were determined after 1,5,10 passages on antibiotic-gradient plates and after 10 subcultures on antibiotic free agar.
Resistant strains selected were characterised for DNA mutations by sequencing gyrA, gyrB, grlA, parC and parE genes.
Results: Frequencies of mutations in *P. aeruginosa* were similar at plasma and ELF peak concentrations for LVX (median: 8×10^{-10} and $<10^{-11}$ at plasma, 4×10^{-11} and $<10^{-11}$ at ELF, LVX 500 mg and LVX 750 mg, respectively) and CIP (median: $<10^{-11}$ and 8.67×10^{-10}, at plasma and ELF, respectively) but higher for PRU (median: 1.25×10^{-8} and 1.01×10^{-7} at plasma and ELF, respectively). In *S. aureus* higher frequencies of mutations were obtained both with CIP (median: 1.11×10^{-7}) and PRU (median: 2.76×10^{-10}) than with LVX ($< 10^{-11}$) at plasma peak concentration.
P. aeruginosa was more prone than *S. aureus* in becoming resistant to fluoroquinolones after multi step selection (58 resistant strains vs 47, *P. aeruginosa* vs *S. aureus*). The lowest number of resistant strains was selected by LVX 750 mg both in *P. aeruginosa* (n = 33) and *S. aureus* (n = 3).
Mutations in gyrA, parC, grlA and gyrB were mostly associated with resistance.
Conclusions: Fluoroquinolones are characterised by a different ability to select for resistance, depending on bacterial species and in vivo drug concentrations. Generally, ELF concentrations selected for less resistant strains than plasma concentrations. Globally, LVX selected for less resistance than comparators both in *P. aeruginosa* and *S. aureus*.

P1047 Effects of β-lactams on mixed cultures of common respiratory isolates as an approach to treatment effects on nasopharyngeal carriage

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Objectives: To explore β-lactam effects on the evolution over time of a bacterial load containing common pharyngeal isolates.
Methods: A computerised two-compartment pharmacodynamic model simulating free serum concentrations obtained with amoxicillin (AMX) 875 mg tid, amoxicillin/clavulanic acid (AMC) 875/125 mg tid and cefditoren (CDN) 400 mg bid regimens over 24 h was used. Strains and MICs (mg/l) of AMX, AMC and CDN were: *S. pyogenes* (0.03, 0.03 and 0.015), penicillin-resistant *S. pneumoniae* (PRP; 2, 2 and 0.25), a β-lactamase positive *H. influenzae* (BL+; >16, 2 and 0.06) and a β-lactamase positive AMC-resistant *H. influenzae* (BLPACR, >16, 8 and 0.06). A mixture of identical 1:1:1:1 volumes of bacterial suspensions of each strain were prepared yielding an inoculum approx. 4×10^6 cfu/ml. Antibiotic concentrations were measured both in bacteria-free antibiotic simulations (SBF) and in antibiotic simulations with the mixed inocula (SMI) and T>MIC calculated.
Results: T>MIC (% dosing interval) and mean % initial inocula reduction (%IIR; cfu/ml) are shown in the Table.

	CDN 400 mg bid			AMX 875 mg tid			AMC 875/125 mg tid		
	T>MIC		% IIR	T>MIC		% IIR	T>MIC		% IIR
	SBF	SMI		SBF	SMI		SBF	SMI	
S. pyogenes	84.3	79.0	E	99.9	24.9	92.8	99.9	99.9	E
PRP	24.0	25.6	94.1	43.2	17.7	41.8	40.8	32.8	78.4
BL+	57.4	58.0	E	0.0	0.0	+66.6	40.8	32.8	+20.1
BLPACR	57.4	58.0	E	0.0	0.0	+2.5	4.5	4.6	+215.1

E = eradicated (cfu/ml below limit of detection). Positive values mean regrowth.

β-lactamase production decreased AMX concentrations and T>MIC against PRP or *S. pyogenes*, and eradication was precluded. The presence of clavulanic acid countered this effect, and *S. pyogenes* was eradicated (but not BL+ and PRP). CDN resistance to TEM β-lactamase avoided this effect, and eradicated *S. pyogenes*, BL+ and BLPACR, and reduced in 94.1% *S. pneumoniae* counts.
Conclusions: Co-pathogenicity seems to be gradual since calvulanic acid countered this effect for strains very susceptible to AMX as *S. pyogenes* but not for strains with AMX MICs values in the limit of susceptibility as PRP. There is a potential therapeutic advantage for β-lactamase resistant cephalosporins with high intrinsic activity against streptococci.

P1048 Plasma protein binding of anidulafungin is similar to other major Echinocandins

P. Inskeep, J. Lin (Groton, US)

Introduction: Anidulafungin is one of three echinocandins approved in the USA for intravenous treatment of systemic fungal infections. Early reports, using ultracentrifugation methods relying on total radioactivity, indicated that protein binding of anidulafungin in human plasma (84%) was substantially lower than independently determined values of protein binding for caspofungin (97%) or micafungin (99%). Similar differences were observed for rat and dog plasma. Subsequent to the initial protein binding study with anidulafungin, a non-enzymatic intramolecular rearrangement of anidulafungin when incubated in plasma was observed. This product retains the radiolabel and thus would not be distinguishable from anidulafungin when total radioactivity is measured in the protein-free fraction from ultracentrifugation.
Objectives: To accurately determine protein binding at 37°C of unlabeled anidulafungin in rat, dog, monkey, and human plasma using dialysis and an LC/MS/MS method that is specific for parent.
Results: Using dialysis and the specific LC/MS/MS assay, greater than 99% of plasma anidulafungin was observed to bind to protein across the initial concentration range of 1.0 to 10 ug/mL for all 4 species (and 100 ug/mL in monkey plasma). Thus, all three major echinocandins appear to be extensively bound to plasma proteins. In addition, the amount of parent anidulafungin decreased during the incubation period suggesting that non-binding degradation products of anidulafungin were formed.
Conclusions: Plasma protein binding of anidulafungin is similar to other major echinocandins. These studies underscore the importance of using specific analytical methods, rather than total radioactivity, in assessing the protein binding of macromolecules having the potential for non-enzymatic rearrangements.

P1049 Lack of anidulafungin interactions with CYP enzymes and transporters in in vivo and in vitro systems

P. Inskeep, R. Walsky, B. Feng, S. Campbell (Groton, US)

Introduction: Anidulafungin (ANID) is one of three echinocandins approved in the USA for intravenous treatment of systemic fungal infections. Patients with severe fungal infections treated with echinocandins are usually hospitalised and on multiple drug regimens. The lack of drug-drug interactions (DDI) of and by ANID with other agents minimises the need for dose adjustments during polytherapy.
Objectives: Use in vivo and in vitro systems to assess the DDI potential of ANID.
Methods: Pooled human liver microsomes and purified CYP2C8 were used to investigate CYP interactions. In vivo studies in rats and monkeys were used to assess the potential for CYP induction. Transporter-transfected HEK cells were used to investigate the potential for ANID to interact with hepatic transporter systems.
Results: In pooled human liver microsomes, ANID did not inhibit CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6 or CYP3A (IC50>17.5 uM), and a slight inhibition (12.0 uM) of CYP2C8 (amodiaquine N-deethylase activity) was observed. However, using purified recombinant human CYP2C8, no inhibition (IC50>17.5 uM) was observed (rosiglitazone N-demethylase activity). In a 4-week study in cynomolgus monkey dosed at 0, 2, 5, or 30 mg ANID/kg/day, no changes in total hepatic cytochrome P450 content were observed. In a 4-week study in Fischer 344 rats dosed at similar targeted doses, no increases occurred in hepatic enzymatic activity assessed by total P450 content, 7-ethoxyresorufin O-deethylase, benzphetamine N-demethylase (36% decrease in high dose male rats), and erythromycin N-demethylase activities. These studies demonstrate a low potential for ANID to induce hepatic enzyme activities. The potential for anidulafungin DDI via interaction with 3 major hepatic transporter systems was also investigated. ANID was not a substrate for organic anion transporting polypeptides hOATP-B, hOATP-C, and hOATP-8 across the concentration range of 0.01 to 100 uM. ANID did not inhibit uptake of probe substrates for hOATP-B or hOATP-C across the concentration range, and only showed slight inhibition of hOATP-8 (approx. 20%) at a concentration of 10 uM.
Conclusion: Given that circulating plasma concentrations of anidulafungin in humans at steady state following the 100 mg loading dose and 50 mg/day treatment are in the range of 1 to 3 uM, these results indicate a very low probability of DDI through CYP or OATP transporter interactions by anidulafungin.

P1050 The antibacterial activity of PZ-601 (SMP-601) against MRSA studied in an in vitro pharmacokinetic model

A. Noel, K. Bowker, A. MacGowan (Bristol, UK)

Objectives: PZ-601 (PZ) is a new injectable carbapenem with in vitro activity against MRSA ⩽2 mg/L. We studied the antibacterial activity of PZ at simulated human serum concentrations against 5 strains of MRSA, PZ MIC range 0.09–2 mg/L, in an in vitro pharmacokinetic (pK) model.
Methods: A single compartment open dilutional in vitro pK model was used. 5 strains of MRSA were tested: representative strains of UKEMRSA 15 (PZ MIC 0.38 mg/L), UKEMRSA 16 (PZ MIC 0.19 mg/L), the Michigan strain VISA (PZ 0.75 mg/L) and two MRSA

with MIC values on the low (PZ MIC 0.09 mg/L) and high (PZ MIC 2 mg/L) ends of the MIC distribution. PZ concentrations simulated were Cmax of 35 mg/L, $t_{\frac{1}{2}}$ 1.5 h with dosing 12hly for 48 h. The inoculum was 10^6 CFU/ml. Emergence of resistance was assessed by population analysis profiles.
Results: The antibacterial effect was – PZ reduced MRSA count by 2.3–4.3 logs at 24 h and >4 logs at 48 h. Strain kill kinetics were similar across the range of MICs tested. No resistance was detected.
Conclusion: PZ-601 at simulated human serum concentrations had a marked antibacterial effect against MRSA strains MIC ⩽2 mg/L

	Strain				
	33922	36895/ EMRSA16	27706/ EMRSA15	19898 VISA	33820
PZ MIC (mg/L)	0.09	0.19	0.38	0.75	2.0
log change in viable count at					
6 h	–2.1	–3.7	–2.9	–3.1	–2.4
12 h	–2.5	–3.7	–2.5	–3.5	–2.3
24 h	–2.4	–3.7	–2.3	–4.3	–2.3
36 h	–4.2	–4.4	–4.5	–4.3	–4.2
48 h	–4.2	–4.4	–4.5	–4.3	–4.2

P1051 The antibacterial activity of telavancin and vancomycin against UK EMRSA 15 and 16 strains studied in an in vitro pharmacokinetic model

A. Noel, K. Bowker, S. Tomaselli, A. MacGowan (Bristol, UK)

Objective: Telavancin (tela) is an injectable new semi synthetic lipoglycopeptide antibiotic with broad activity against Gram-positive pathogens including MRSA. It has a double mechanism of action on the cell membrane and wall resulting in bactericidal activity. We used an in vitro pharmacokinetic (pK) model to simulate serum free drug concentrations of tela and vancomycin (vanco), and studied their antibacterial effect against representative strains of UK EMRSA 15 and 16.
Methods: A single chamber dilutional in vitro pK model was used to simulate serum free drug concentrations. Tela Cmax 10 mg/L, $t_{\frac{1}{2}}$ 8 hr, 2 doses over 48 h; vanco Cmax 14 mg/L, Cmin 3.5 mg/L, 4 doses over 48 h. Two strains of UK EMRSA 15 and 16 were used (both tela MIC 0.19 mg/L). Experiments were performed in triplicate at an initial inoculum of 10^6 CFU/ml. Antibacterial effect was measured by log change in viable count at 6 h (d6), 12 h (d12), 24 h (d24) and 48 h (d48). The maximum kill was recorded (dmax) and the area-under-the-bacterial-kill curve between 0–24 h (AUBKC24) and 0–48 h (AUBKC48) calculated.
Results: The killing kinetics of both strains were similar, therefore the antibacterial effect measures were pooled for tela and vanco, and compared.
Conclusion: Tela produced significantly greater early killing of EMRSA 15 and 16 than vancomycin (d6, d12). The maximum kill was also greater (dmax) as was the antibacterial effect in the first 24 h (AUBKC24).

	tela	vanco	p
d6	–3.8±0.2	–2.7±0.8	0.008
d12	–4.0±0.3	–3.3±0.3	0.034
d24	–1.9±0.4	–2.4±0.2	0.134
d48	–1.7±0.5	–1.7±1.0	0.960
dmax	–4.0±0.1	–3.2±0.1	0.005
AUBKC24	21.5±6.9	33.8±3.2	0.05
AUBKC48	62.5±17.7	85.5±10.5	0.12

P1052 Inoculum effect of β-lactams and killing curves determination in AmpC-producing (DHA-1) *Klebsiella pneumoniae* isolates

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Objectives: An inoculum size is a critical point in treatment of infections caused by extended-spectrum β-lactamase- and AmpC-producing bacteria. A treatment failure was described in many cases of infections due to these bacteria. An inoculum effect determined by standard dilution method is not an appropriate model of the infection process in organism. The aim of this study is to compare the inoculum effect determination on β-lactams by a standard microdilution method and the description of dynamic of bacterial population of AmpC-producing *Klebsiella pneumoniae* after addition these antibiotics.
Methods: Four DHA-1 producing *Klebsiella pneumoniae* strains (two inducible and two with high level of expression) were used in this study. MIC and inoculum effect was determined by a classical microdilution broth method (CLSI). Dynamic of bacterial population – bacterial count was measured by direct plating samples after addition antibiotic in brain-heart infusion.
Results: Using standard inoculum (10^5 CFU per ml), the strains producing inducible AmpC were determined as non-resistant to cefotaxime (CTX) (MIC = 1 mg/l), ceftazidime (CAZ) (MIC = 4 mg/ml) and cefepime (FEP) (MIC = 2 mg/l) according to EUCAST criteria. When the inoculum was 10^6 CFU/ml, the strains were resistant to all tested β-lactams (e.g. MIC of CTX was 256 mg/ml, MICs of CAZ and FEP were 1024 mg/ml). The strain with a high-level expressed AmpC was not resistant only to cefepime (MIC = 2 mg/l). MIC of CTX was 64 mg/l and of CAZ 128 mg/l. Killing curves showed different times required for an inactivation of antibiotics. For the strains producing inducible AmpC, decreasing count of bacteria were observed in CTX for 6 hrs in a concentration of 1 mg/l, at least for 7 hrs in a concentration of 2 mg/l, in CAZ for 3 hrs in a concentration of 1 mg/ml, for 6 hrs in a concentration of 8 mg/l and in FEP for 3 hrs in a concentration of 1 mg/l and at least for 7 hrs in a concentration of 8 mg/l.
Conclusions: This work showed that the time required for hydrolyzing antibiotics by β-lactamases under the MIC is shorter than the time recommended for MIC reading by standard method. Therefore the data obtained using microdilution method do not exactly described the situation in the organism and the inoculum effect could be misinterpreted due to lack of antibiotic in a well of microtiter plate.
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P1053 In vitro enrichment of resistant *Staphylococcus aureus* at daptomycin subtherapeutic concentrations within the mutant selection window: effect of treatment duration

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Objective: To study time-dependent enrichment of resistant sub-populations and concomitant loss in susceptibility of *S. aureus*, ten-day treatments with daptomycin (DAP) were simulated in vitro.
Methods: *S. aureus* ATCC 43300 (MICDAP 0.2 mg/L) was exposed to once-daily dosing of DAP at subtherapeutic ratios of area under the curve (AUC) to the MIC of 32 and 64 h, which have been reported recently to result in maximal selection of resistant mutants. Susceptibility (culture MIC) and bacterial growth on agar plates containing 2× and 4×MIC were tested daily. To provide an integral presentation of the time course of mutants grown on DAP-containing plates, areas under the bacterial mutant kinetic curves (AUBMCs) were calculated from the start of treatment to the 3rd (AUBMC3), 5th (AUBMC5) and 10th day (AUBMC10) of treatment.
Results: With both dosing regimens, DAP-resistant *S. aureus* were enriched on the third day. Further enrichment occurred gradually, without abrupt increases in mutant numbers. By the end of each treatment, numbers of mutants resistant to 2× and 4×MIC of DAP were comparable to those of susceptible organisms, but they were not completely replaced

by DAP-resistant mutants. Similar patterns were inherent in the time course of susceptibility of DAP-exposed cultures, with 16- and 8-fold increases in the MIC by the end of treatment at AUC/MIC of 32 and 64 h, respectively. This gradual enrichment of resistant mutants and concomitant loss in susceptibility were confirmed by the AUBMC analysis. At AUC/MIC of 32 h, production of mutants resistant to 4×MIC was reflected by a 2.9 times greater AUBMC10 than AUBMC5 and by a 2.2 times greater AUBMC5 than AUBMC3. At AUC/MIC of 64 h, AUBMC10 was 3 times greater than AUBMC5 and AUBMC5 was 2.1 times greater than AUBMC3.

Conclusions: The time-resistance relationships established in this study: (1) allow prediction of subsequent enrichment of DAP-resistant mutants and concomitant loss in susceptibility after long-term treatments based on data obtained in shorter treatments, and (2) show that relatively minimal early expressions of resistance seen in short treatments are precursors of more resistance that occurs with longer DAP treatments.

P1054 Daptomycin pharmacodynamics against *Staphylococcus aureus* hemB mutants expressing the small colony variant phenotype

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Objectives: *S. aureus* small colony variants (SCVs) are slow-growing morphological variants of *S. aureus* that have been associated with poor clinical response to prolonged antimicrobial therapy. Recently, we determined that vancomycin bactericidal activity is attenuated against genetically defined *S. aureus* mutants displaying the SCV phenotype. Limited data exist on the killing activity of daptomycin (DAP) against SCVs. In this study, the bactericidal activity & pharmacodynamics (PD) of DAP were evaluated against site-directed hemB mutants (hemB::ermB) displaying the SCV phenotype.

Methods: The wild-type meticillin-resistant *S. aureus* strain COL & the meticillin-susceptible strain Newman, as well as their corresponding hemB mutants with SCV phenotype, Ia48 & III33, were used for these studies. MICs were performed in quadruplicate as per CLSI. Log phase time kill experiments were performed in duplicate for each strain at 10^6 CFU/ml at increasing DAP concentrations (0, 0.5, 1, 2, 4, 8, 16, 32, 64 times the MIC) over 24 h. PD analysis was performed using an integrated area approach for reduction in area under the CFU versus time curve from 0 to 24 h, Log10(AUCdrug/AUCgrowth control) versus DAP concentration:MIC which was fit to a Hill-type pharmacodynamic model.

Results: MICs for DAP against COL, Newman, Ia48, and III33 were 1.0, 1.0, 2.0 & 2.0 mg/L, respectively. DAP exhibited concentration dependent, bactericidal activity against both *S. aureus* phenotypes. Against the wild type strains COL and Newman exposure to DAP at concentrations ⩾4.0 x MIC achieved 99.9% kill by 6 h. Values >4.0 log10 CFU/ml reductions occurred at 4 h exposed to ⩾8.0 x MIC of DAP. Against both mutants, 99.9% kill was achieved for DAP ⩾16 x MIC in ⩽8 h. Maximal bacterial reductions for normal phenotype/hemB mutant COL/Ia448 & Newman/III33 at 24 h at maximal exposure were –5.4/–5.0 –5.5/5.0 log10 CFU/ml. Maximum likelihood parameter estimates for reduction in area for Emax for COL/Ia48 & Newman/III33 were 5.8/4.6 & 5.6/4.5. PD model fits describing the concentration-effect relationship for DAP vs. all strains were excellent ($r^2 \geqslant 0.97$).

Conclusion: *S. aureus* SCVs represent a major therapeutic challenge. DAP achieved rapid and sustained bactericidal activity against both *S. aureus* hemB mutants tested displaying the SCV phenotype. This study may have implications for the optimal therapy of persistent and difficult-to-treat infections due to *S. aureus* SCVs.

P1055 In vitro adsorption and release of antibiotics from cancellous bone

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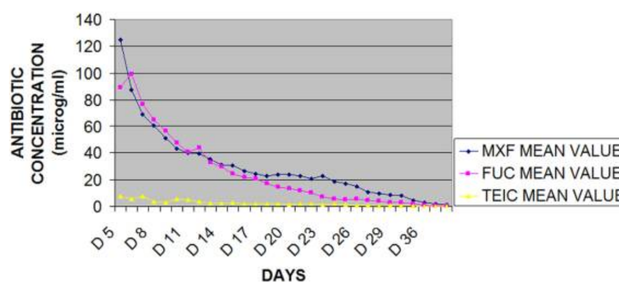
Objectives: Drug delivery systems have become of significant importance in the contemporary management of chronic bone infections. Several antibiotics have been studied for their release patterns from cancellous bone. We report the adsorption and elution profile of human cancellous bone impregnated with moxifloxacin (MXF), teicoplanin (TEIC) and fucidic acid (FUC).

Methods: ~1 g of processed cancellous bone was compressed into a wire-mesh cylinder made of stainless steel and impregnated with 5 ml of antibiotic solution (100 mg/ml, active product ingredient) for 1 hour at room temperature. Five replicates for each antibiotic were incubated separately at 37 oC in 5 ml of Mueller Hinton broth and transferred daily into a new elution tube for 42 days. The amount of antibiotic eluted in broth was assessed by a microbiological assay (agar diffusion using *Bacillus subtilis* ATCC 6633 for MXF and TEIC and *Corynebacterium* 404 CIP 5216 for FUC).

Results: High concentrations of MXF and FUC were measured on the first 5 days (>100 μg/ml) with a rapid decline until day 10 (mean value ~50 μg/ml), showing a steady state curve thereafter. On day 20 the MXF concentration remained significant (~25 μg/ml), whereas the amount of FUC decreased to ~13 μg/ml. On day 30 MXF concentration maintained a value of ~10 μg/ml as opposed to FUC concentration (~2.9 μg/ml). By day 40 the MXF level was reduced to ~2 μg/ml. On the contrary, very low levels of TEIC were measured after the first 4 days (<10 μg/ml).

Conclusion: After an initial burst of release in the first 5 days, MXF attained high concentration in the elution fluid for up to 4 weeks. FUC had a similar release pattern reaching the lowest values in less time. TEIC was found to have an inferior elution profile against the other two antibiotics. Cancellous bone can be used as a carrier of antibiotics for local delivery. The implications of these findings need to be examined in the clinical setting.



Antibiotic elution profile.

P1056 Hypoxia induces antibiotic resistance in *Pseudomonas aeruginosa*

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Objectives: Cystic Fibrosis (CF) is an autosomal recessive disorder with a frequency in Ireland of 1/1461 live births. Pulmonary *Pseudomonas aeruginosa* infection contributes significantly to morbidity and mortality in CF. Within the CF lung biofilm *P. aeruginosa* is exposed to hypoxic conditions and is often found to be multiresistant to various antibiotics. In this study we investigated the influence of hypoxia on antibiotic resistance in *P. aeruginosa*.

Methods: *P. aeruginosa* was incubated in cation adjusted Mueller-Hinton broth for 6 h under either ambient normoxic (21% oxygen) or hypoxic conditions (1% oxygen). IC50 values for chloramphenicol, ciprofloxacin hydrochloride, novobiocin, tetracycline and tobramycin were determined after incubation of *P. aeruginosa* in increasing antibiotic concentrations in either normoxia or hypoxia. IC50 values were calculated as changes in optical density measurements (OD610). To identify potential molecular mechanisms underlying resistance development, expression levels of the 4 major multidrug efflux pumps of *P. aeruginosa* (mexAB-OprM, mexCD-OprJ, mexEF-OprN) were investigated. After incubation in hypoxia bacterial mRNA was isolated using phenol chloroform extraction and reverse transcribed. Expression levels were measured by real time PCR with primers amplifying segments from mexA, mexC, mexE cDNA.

Results: Incubation in hypoxia for 6 h significantiy increases IC50 values for ciprofloxacin HCl (p = 0.038) and tobramycin (p = 0.006). IC50 values for chloramphenicol (p = 0.076) and novobiocin (p = 0.083) were increased without reaching significance. Hypoxia did not affect IC50 values for tetracycline. Preliminary analysis of mRNA expression levels for mexA, mexC and mexE showed no difference between normoxic and hypoxic conditions.
Conclusion: Short term incubation in hypoxia increases antibiotic resistance in *P. aeruginosa*. We speculate that hypoxia within the CF lung could be an important stimulus for the development of antibiotic resistance.

P1057 Evaluation of vancomycin and daptomycin against MRSA and hVISA in an in vitro PKPD model with simulated endocardial vegetations

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Objectives: Historically vancomycin (VAN) has been the primary treatment for infections caused by meticillin-resistant *Staphylococcus aureus* (MRSA). However, continued selective pressure has led to non-susceptible strains including hVISA. Infections with hVISA are associated with prolonged bacteraemia and VAN failure and are often high bacterial load infections. Our own study of hVISA at the Detroit Medical Center (DMC) from 2003–2006 demonstrated a prevalence of 8.3%. The objective of this study is to evaluate VAN and daptomycin (DAP) against VAN susceptible MRSA and hVISA in a PK/PD simulated endocardial vegetations (SEV) model.
Methods: Five clinical isolates were used, MRSA 494, MRSA 67, hVISA(s) 1720, 2295 and 1629. All isolates were obtained from the DMC. The hVISA strains were confirmed by population analysis profile ratio with Mu3 as the control strain. Simulated doses of VAN 1 g Q12 h (Cmin 5–10 mg/L) and 2 g Q12 h (Cmin 15–20 mg/L), and DAP 6 mg/kg Q24 h (Cmax 95.7 mg/L), 10 mg/kg Q24 h (Cmax 129.7 mg/L), and 12 mg/kg Q24 h (Cmax 164.8 mg/L) were used in the PK/PD SEV model over 72 h in duplicate. Changes in bacterial CFU/g at 72 h were compared by ANOVA with Tukey's post-hoc test. $P \leqslant 0.05$ was considered significant.
Results: VAN MIC was 0.5 mg/L for MRSA 494, MRSA 67, and hVISA 1720; 1 mg/L for 2295; and 2 mg/L for 1629. DAP MIC values were 0.125 for MRSA 67 and hVISA 1720 and 0.25 mg/L for MRSA 494, hVISA 1629, and hVISA 2295. Against both MRSA strains DAP 10 and 12 mg/kg killed to detection limits (> 7 log10 CFU) by 24 h and 6 mg/kg by 48 and 56 hours (average T99.9 for 6 mg/kg – 6.2 h; 10 mg/kg – 2.7 h; 12 mg/kg – 2.4 h) whereas VAN achieved a maximum kill of 2–3 log10 CFU/g by 72 h (P<0.01). Against hVISA strains, VAN only reduced the bacterial load by 1–1.5 log10 CFU/g over 72 h regardless of dose exposure, whereas DAP achieved rapid bactericidal kill (average T99.9 for 6 mg/kg – 10 h; 10 mg/kg – 5.6 h; 12 mg/kg – 4.8 h) to detection limits of 2 log10 CFU/g (P< 0.01).
Conclusion: Overall, DAP achieved rapid and effective kill against both MRSA and hVISA. VAN however, displayed slow and minimum kill against MRSA at high inoculum and displayed minimal to no activity versus hVISA regardless of high dose exposure. Further evaluation of high-dose DAP is warranted.

P1058 Intracellular activity of antibiotics against a stable small colony variant of *Staphylococcus aureus* isolated from a CF patient in model Calu-3 human airway epithelial cells

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Objectives: Persistence of *S. aureus* infections in CF may be related to the presence of small colony variant (SCV) variants and of intracellular bacteria. Using a stable thymidine-auxotrophic mecA negative SCV of *S. aureus* isolated from a CF patient, we showed that most antibiotics (AB) act only poorly on intracellular forms in a model of human THP-1 macrophages (ICAAC 2007, abstr. A1437). We have now compared the activity of commonly used (gentamicin, rifampicin, vancomycin, moxifloxacin) and newly developed AB (linezolid, tigecycline, oritavancin) against this SCV isolate using Calu-3 cells as a model of airway epithelium.
Methods: MICs were determined in MH broth after 48 h incubation. Intracellular activities (IA) were measured as the change in post-phagocytosis inoculum [delta log CFU] after 24 h or 96 h in Calu-3 cells (normal bronchial epithelial cells) incubated with ABs at extracellular concentrations (Ce) corresponding to their respective peak (Cmax), free peak (fCmax), trough (Cmin) and free trough (fCmin) in plasma when administered at standard doses.
Results: Over 24 h, the intracellular inoculum remained constant (–0.14 ± 0.01 log CFU with 10 mg/L lysostaphin to prevent extracellular growth). All AB showed a concentration-dependent effect, with a significant inoculum decrease at Cmax for TGC, LNZ and RIF, fCmax for MXF, Cmin for ORI low dose, and fCmin for GEN, VAN and ORI high dose. At 96 h, the activity of all drugs (except LNZ and TGC) markedly progressed at Cmax, approaching or reaching a bactericidal effect, while regrowth was observed at Cmin for all drugs but VAN, MXF and RIF.

AB	MIC (mg/L)	delta log CFU measured at 24 h for Ce (mg/L) corresponding to							
		Cmax		fCmax		Cmin		fCmin	
		Ce	IA	Ce	IA	Ce	IA	Ce	IA
TGC	0.125	1	–0.54±0.05	0.2	0.17±0.04	0.13	0.18±0.12	0.026	0.55±0.14
LNZ	2	16	–0.98±0.07	8	–0.19±0.04	4	–0.16±0.08	2	0.21±0.09
RIF	0.0005	18	–0.59±0.08	2.7	–0.28±0.08	1.2	–0.23±0.11	0.18	–0.14±0.09
MXF	0.125	4	–1.74±0.18	2	–1.34±0.05	0.4	–0.20±0.08	0.2	0.17±0.02
GEN	0.125	18	–1.72±0.10	18	–1.72±0.10	1.5	–0.62±0.11	1.5	–0.62±0.11
VAN	0.5	50	–1.59±0.01	22.5	–1.28±0.04	10	–0.93±0.12	4.5	–0.89±0.09
ORI 200 mg	0.015[a]	32	–1.91±0.02	4	–1.27±0.05	2	–0.85±0.05	0.5	–0.22±0.02
ORI 800 mg	0.015[a]	128	–2.53±0.53	16	–1.61±0.05	16	–1.81±0.05	2	–0.85±0.05

[a] In the presence of 0–0.002% P80.

Conclusion: Prolonged incubation with high concentrations of bactericidal drugs is needed to durably act on intracellular SCVs, suggesting the importance of AB selection and PK/PD optimisation to avoid failure in eradicating these bacteria in CF patients.

P1059 Activity of antibiotic combinations towards intracellular small colony variants of *Staphylococcus aureus* in a model of THP-1 macrophages

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Objectives: In a model of human THP-1 macrophages intracellular infection, small colony variants (SCV)s of *S. aureus* have been shown to be poorly susceptible to most antibiotics (ICAAC 2007, A1437), except ORI, MXF or RIF (–2 log CFU in 24 or 72 h). In the present study, we have examined whether combining these drugs could further enhance intracellular efficacy in comparison with conventional associations (RIF-OXA, RIF-VAN, RIF-FA) as comparators.
Methods: We used a stable thymidine-auxotrophic mecA negative SCV of *S. aureus* isolated from a cystic fibrosis (CF) patient. MICs were determined in MH broth after 48 h incubation. Intracellular activities were measured as the change in post-phagocytosis inoculum [delta log CFU] after 24 h or 72 h of incubation with antibiotics at extracellular concentrations corresponding to their respective serum peak concentration (Cmax) when administered at standard doses.
Results: Combination of RIF with OXA, VAN, FA, or MXF was indifferent (same activity as RIF alone at 24 h, slight reduction at 72 h). In contrast, combination of ORI with RIF or MXF resulted in synergistic effects both at 24 h and 72 h (higher activity than observed for the most active drug given alone), with RIF-ORI reaching the limit of detection at 72 h.
Conclusion: The data suggest that combining oritavancin with other bactericidal antibiotics may prove useful for eradicating intracellular SCVs, and plead for the further evaluation of such combinations in in vivo pertinent models of recurrent staphylococcal infections that imply SCVs.

Antibiotic(s)	MIC (mg/L)	Cmax (mg/L)	Delta log CFU from initial inoculum at	
			24 h	72 h
Oxacillin [OXA]	0.125	64	−0.06±0.04	−1.31±0.06
Vancomycin [VAN]	0.5	50	−0.11±0.04	−1.35±0.08
Fusidic acid [FA]	0.03	30	−0.33±0.07	−0.87±0.04
Rifampicin [RIF]	0.0005	18	−1.40±0.18	−3.39±0.18
Moxifloxacin [MXF]	0.125	4	−1.20±0.05	−2.53±0.04
Oritavancin [ORI]	0.015[a]	25	−2.53±0.10	−2.98±0.11
RIF–OXA			−1.28±0.05	−2.81±0.01
RIF–VAN			−1.42±0.04	−2.75±0.01
RIF–FA			−1.06±0.01	−2.44±0.11
RIF–MXF			−1.22±0.06	−2.95±0.07
RIF–ORI			−3.19±0.10	>5[b]
MXF–ORI			−3.26±0.13	−4.18±0.16

[a]Determined in the presence of 0.002% P80.
[b]Below the limit of detection (−5 log).

P1060 Identification of a *Staphylococcus aureus* strain with increased intracellular growth and reduced intracellular susceptibility to gentamicin

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Objectives: The persistence and recurrence of many *S. aureus* infections (such as endocarditis) has been ascribed to its intracellular character. Our aim was to examine the intracellular development of *S. aureus* strains differing by their hemolysin (hl) production as well as their intracellular response to antibiotics, using both phagocytic (THP-1) and endothelial cells (HUVEC).
Methods: Intracellular growth was measured by the change in CFU (Delta log /mg prot.) recorded 24 h after infection. Controls were maintained with gentamicin (1 x MIC) to prevent the development of extracellular bacteria. Confocal microscopy was performed with bacteria tagged with fluorescein isothiocyanate, and vital staining of lysosomes using LysoTracker® Red DND 99.

Strain	Characteristics	Gentamicin MIC[a] (μg/mL)	Intracellular growth in THP-1	Gentamicin concentr. (in × MIC) allowing to reach a static effect	
				Intracell.	Extracell.
8325-4	Laboratory strain cured of all prophages.	0.2	0.2±0.1	2.3 × MIC	1.5 × MIC
DU5942	Mutant derived from 8325-4. Disrupted for gamma-hemolysin (hlg)	0.3	1.9±0.1*	5.0 × MIC	1.3 × MIC
DU5942-M1	Mutant derived from 8325-4. Disrupted for hlg and complemented with pCU1-*hlg+*	0.3	1.8±0.1*	5.0 × MIC	1.3 × MIC
DU5938	Mutant derived from 8325-4. Disrupted for alpha- (hla), beta- (hlb), and gamma- (hlg) hemolysins	0.2	0.3±0.1	–	–

n = 3. *p < 0.001 (ANOVA)
[a]Microdilution in Mueller-Hinton broth pH 7.4.

Results: *S. aureus* DU5942 showed a more important intracellular growth than the other strains (also observed in HUVEC). This was not due to hlg disruption, since it was also observed for the complemented strain DU5942-M1 but not for the multidisruptant strain DU5938. Dose-effects studies with gentamicin showed a similar response for all tested strains extracellularly. In contrast, DU5942 and its M1 mutant were less sensitive to gentamicin than 8325–4 intracellularly, exposure to higher extracellular concentrations being required to prevent intracellular growth. No difference was seen between strains for other antibiotics (oxacillin, vancomycin or telavancin). Confocal microscopy showed that, after 24 h of infection, all strains were confined in lysosomes in THP-1 macrophages. In endothelial cells, most bacteria colocalised with lysosomes but a small number appeared free in the cytoplasm.
Conclusion: We identified a strain with high capacity of intracellular growth in both professional phagocytes and endothelial cells. This effect seems to be related to a lower intracellular susceptibility of the strain to gentamicin but not to its hl status.

P1061 Quantitative determination and pharmacokinetics of oritavancin in rabbit serum and tibia

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Objectives: Oritavancin is a novel lipoglycopeptide characterised by its activity against Gram-positive organisms, including vancomycin-resistant staphylococci and enterococci, and its long half-life. It is under preclinical evaluation for the treatment of osteomyelitis, an acute or chronic bone infection predominantly caused by staphylococci and requiring prolonged parenteral antibiotic administration. Given the importance of the rabbit model of chronic osteomyelitis in predicting clinical efficacy, we report here pharmacokinetic parameters in rabbit serum and tibia.
Methods: Rabbits were administered a single intravenous (IV) dose of oritavancin at 20 mg/kg. They were sacrificed at 7 time points ranging from 5 minutes to 7 days (n = 3/time point), a blood sample was collected and the tibiae were harvested and ground to a powder. Oritavancin was extracted from serum by the precipitation of serum proteins with acidified acetonitrile. Given the difficulties in extracting the oritavancin from bone, an indirect method of analysis was sought whereby the concentration of oritavancin was determined through the detection of a product of acidic degradation, 4-(4-chlorophyl)benzyl-4-epi-vancosamine (CBV). A 50 mg fraction of the tibia powder was acidified with 0.5 ml of 1N HCl and incubated for 5 minutes at 70 degrees Celsius. After centrifugation, the supernatant was transferred and the pellet treated two times under the same conditions. CBV in the pooled supernatants was concentrated by solid phase extraction. The quantitative determinations of oritavancin and CBV relied on liquid chromatography/mass spectrometry (LC/MS).
Results: The dose-response curves of oritavancin in serum and in tibia were linear with a limit of quantitation (LOQ) of 0.0125 mg/L and 0.2 mg/kg, respectively. The serum concentration was 200 mg/L at 5 minutes with a half-life of 2.4 hours, falling below 5 mg/L after 24 hours with a terminal half-life of 40 hours. The maximal concentration of the drug in tibia was 15 mg/kg of bone, reached at 5 minutes and maintained for at least the first 24 hours, and a half-life of 12 days. The calculated area under the curve (AUC) in serum and tibia was 1001 hour x mg/L and 1992 hour x mg/kg, respectively.
Conclusion: Oritavancin rapidly penetrates in osseous tissues and is maintained there at high concentration. It is slowly eliminated, such that its levels are maintained for days, suggesting a potential for the treatment of bone infection.

P1062 In vivo selection of carbapenem-resistant *Pseudomonas aeruginosa* following sub-optimal dosing

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Objective: We have previously demonstrated *P. aeruginosa* (PA) resistance emergence due to sub-optimal carbapenem exposures in an in-vitro infection model (Antimicrob Agent Chemother 05), but the in-vivo relevance of the observations is not well established. We examined the impact of a sub-optimal carbapenem exposure on PA resistance emergence in a neutropenic murine pneumonia model.
Methods: Female Swiss-Webster mice (22–26g) were rendered neutropenic by 2 doses of cyclophosphamide on day-4 and -1; transient nephrotoxicity was induced by uranyl nitrate on day-2. Anesthesised animals were infected with approximately 10^6 CFU of PA [a mixture

of wild-type (WT) ATCC 27853 and its isogenic porin deleted (OprD−) mutant (MIC of 1 mg/l and 4 mg/l, respectively) in a 1000:1 ratio] intratracheally under laryngoscopic guidance. Serum TNF-alpha and IL-6 were measured by ELISA 16 hours after infection. Pharmacokinetics of meropenem in infected animals was determined by a single dose study. Ten animals were treated with either IP meropenem 400 mg/kg every 8 hours or placebo. Treatment was given 2 hours after infection for 4 days. Quantitative assessment of bacterial burden [total and those with reduced susceptibility (⩾3× MIC)] in animal lung tissues was performed at baseline, upon death or at the end of experiment.
Results: Both serum TNF-alpha and IL-6 were found to be significantly elevated in infected animals, compared to controls (p<0.01). The meropenem dose used (400 mg/kg) resulted in a Cmax of 354.1 mg/l and terminal t1/2 of 18.9 minutes. The corresponding T>MIC for the WT and OprD-mutant were 33% and 26%, respectively. Meropenem therapy offered a significant survival benefit at day 4 (100% vs 0%, p < 0.01), but complete replacement of bacterial population by the OprD-mutant in lung tissue was observed in 30% of the animals.
Conclusion: Our in-vivo results validated previous in-vitro observations that sub-optimal meropenem exposures might facilitate selective amplification of resistant sub-population(s) in a heterogeneous PA population. Optimal dosing regimen design should aim at preventing resistance emergence during treatment, in addition to clinical benefits.

P1063 Itraconazole-ciprofloxacin interaction on the pharmacokinetics of the intestinal compartment: preliminary results in rats

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Among their pharmacokinetic characteristics fluoroquinolones (FQs) exhibit transintestinal and biliary elimination; moreover, they may interfere with hepatic metabolism. Interactions of FQs with enzymeinhibitors or inducers could be possible during therapy. The inhibitory effects on the hepatic metabolism of Itraconanzole (Itz) should be considered in the kinetics of FQs.
Objectives: To study the effect of Itz on intestinal kinetics of Ciprofloxacin (Cpx) following different administration schedule.
Methods: Wistar rats (weight 174.0+12.5 g, mean+SD) divided in three groups were treated as follows: group 1: Cpx, 15 mg/kg/day, orally administered by gavage for three days; group 2: Cpx two hours after oral administration of Itz (10 mg/kg/day) for three days; group 3: Cpx for 3 days and Itz (10 mg/kg/day) on the 2nd and 3rd day of Cpx administration. Samples of blood, intestinal and hepatic tissues and faeces were collected after three days of treatment, two hours after the last Cpx administration. Cpx concentrations were determined by microbiological assay (agar-well diffusion method, *K. pneumoniae*, Isosensistest agar).
Results: reported in the table as mean+SD
After three days of Itz administration the levels of Cpx increased significantly in liver and faeces (not significantly) while decreased in serum (1/4 positive samples) and intestinal tissues. The administration of Itz for 2 days in rats in treatment with Cpx induced a significant increase of Cpx serum levels, a reduction of intestinal levels and a marked decrease in faeces in comparison to Cpx alone.

Drugs	Serum (mg/L)	Intestinal tissue (mg/kg)	Liver (mg/kg)	Faeces (mg/kg)
Cpx	0.05±0.01 (5/5)	10.4±12.2	0.0±0.0	231.6±279.9
Cpx + Itz 3 days	0.06 (1/5)	4.2±2.7	1.0±0.3**	367.3±264.9
Cpx + Itz 2nd-3rd d	0.2±0.2* (5/5)	7.2±2.4	0.0±0.0	111.8±29.7

*p < 0.05, **p < 0.01 Students t test versus Cpx alone

Conclusions: These preliminary results suggest a possible involvement of Itz on the intestinal secretory mechanisms of Cpx and changes in the enterohepatic circulation. The inhibitory effects of Itz on the Cpx intestinal pharmacokinetics seems different according to timing of Itz administration.
MIUR PRIN 2003-Prot. N. 2003051858_003

P1064 Different effect of pre-treatment with phenobarbital on transintestinal elimination of moxifloxacin and ciprofloxacin: preliminary data

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The fluoroquinolones (FQ) are widely used in clinical practice, and exhibit pharmacokinetic properties such as good oral bioavailability, transintestinal elimination through the gut, and biliary excretion. FQs can interfere with hepatic metabolism and drug interaction with enzyme inducers may occur. Few data are available about intestinal concentrations of FQs and their pharmacokinetics interactions in the gastrointestinal compartement.
Objectives. To study the effect of phenobarbital (Phe, enzyme inducer) on intestinal, hepatic and faecal concentrations of Moxifloxacin (Mxf) and Ciprofloxacin (Cpx).
Methods. 30 Wistar rats (180.2+/-7.8 g weight, mean+SD) were divided in 6 groups and treated as follows: group1: Phe (80 mg/kg/day, i.p.) and Mfx (10 mg/kg/day, po) 2 hours later; group 2: Phe (80 mg/kg/day, i.p.) and Mfx (50 mg/kg/day, po) 2 hours later; group 3: Phe (80 mg/kg/day, i.p.) and Cpx (15 mg/kg/day, po) 2 hours later; group4: Mfx (10 mg/kg/day, po); group 5: Mfx (50 mg/kg/day, po); group 6: Cpx (15 mg/kg/day, po). Samples of blood, intestinal and liver tissues, and faeces were collected after three days, 2 hours after the last FQs administration. Cpx and Mxf concentrations were determined with microbiological method (agar diffusion, *K. pneumoniae*, Isosensitest agar).
Results. Mfx was not detected when administered at 10 mg/kg/day; pre-treatment with Phe induced no modifications. Higher doses of Mxf (50 mg/kg/day) produced good intestinal concentrations (10.3+/-5.5 mg/kg) that increased to 16.3+/-4.3 mg/kg in rats pretrated with Phe. Faecal concentrations of Mfx are similar in the two treated groups (280.0+/-32.1 mg/Kg). Mfx was not detected in serum and hepatic tissue. Cpx: Intestinal concentrations of Cpx were 10.4+/-12.2 mg/kg, when administered alone, and 24.8+/-23.7 mg/kg in rats pretreated with Phe; parallel respective serum levels showed a slight decrease(0.04 and 0.02 mg/L, respectively). Cpx was detected only in liver of rats pretreated with Phe. Faecal concentrations of Cpx were 231.6+/-279.9 mg/kg when administered alone and 87.0+/-44.0 mg/kg in rats pretreated with Phe.
Conclusions. The enzyme inducer Phe produced an increase of the concentrations of Mfx and Cpx in the intestinal tissue. Mfx and Cpx showed different behaviour in the intestinal compartment. Phe can modify the pharmacokinetics of Cpx and, in minor extent, that of Mxf.
MIUR PRIN 2003-Prot. N. 2003051858_003

P1065 In vivo efficacy of moxifloxacin monotherapy against clinical isolates of *B. fragilis* with intermediate susceptibility

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Objectives: Moxifloxacin (MXF) is the only marketed fluoroquinolone with FDA approval for the monotherapy of complicated intra-abdominal infections. The obligate anaerobic bacterium, *Bacteroides fragilis*, is implicated in intra-abdominal infections. In this study, the antibacterial efficacy of MXF against clinical isolates of *B. fragilis* with intermediate susceptibility (4 mg/L) was evaluated using a murine granuloma pouch model. Breakpoints (susceptible ⩽2 mg/L, intermediate=4 mg/L, resistant ⩾8 mg/L) for *B. fragilis* were defined by CLSI.
Methods: Twenty clinical isolates of *B. fragilis* (MIC=4 mg/L) and *B. fragilis* ATCC 25285 (MIC=0.5 mg/L) were used. Pouches for the murine granuloma pouch model were created by injecting 5 mL of air and 0.5 mL of 0.1% croton oil in olive oil under the skin of the back. After removing the air (day 3), a bacterial suspension was injected into the pouch (day 5). Infected mice were treated with MXF

100 mg/kg IV, b.i.d. for 2 days. This dose simulates the AUC of the human 400 mg once-daily MXF IV dosage. To compare the efficacy of MXF with piperacillin/tazobactam (PIP/TAZ), three of the strains (the ATCC 25285 strain and two intermediate isolates) were studied in the pouch model. Mice were treated with PIP/TAZ 400 mg/kg IV, b.i.d., a dose that simulates the human PK/PD driver time above MIC of 3.375 g q.i.d. PIP/TAZ IV therapy.
Efficacy was assessed by the reduction in colony forming units (CFUs) in pouch exudates 48 hours post-infection compared with the infection control.
Results: MXF, 100 mg/kg b.i.d., achieved a CFU reduction of >99.9% in 15/20 intermediate susceptible strains, >99% in 3/20 strains and >90% in 1/20 strains. A CFU reduction of <90% only occurred in 1/20 strains. The comparison of MFX and PIP/TAZ demonstrated similar efficacy against the ATTC strain and the two clinical isolates despite the differences in the breakpoint category (intermediate [MXF] vs susceptible [PIP/TAZ]).
Conclusions: In a murine granuloma pouch model, MXF achieved a CFU reduction of >2 logs against 90% of clinical *B. fragilis* isolates with intermediate susceptibility (4 mg/L). Furthermore, MXF was as effective as PIP/TAZ against two strains that were intermediate for MXF but susceptible to PIP/TAZ.

Sepsis

P1066 Red blood cell deformability in diabetic patients suffering from severe sepsis

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Objectives: Diabetes mellitus and sepsis are accompanied by haemorheological abnormalities, which cause a secondary hyperviscosity syndrome involved in the pathogenesis of microvascular complications. The diabetic patients are more vulnerable to infections and sepsis is a major cause of morbidity and mortality in diabetes. The aim of the present study was to investigate the impairment of RBC deformability on a series of patients with sepsis, diabetes mellitus and diabetic patients suffering from severe sepsis.
Methods: Forty patients with severe sepsis, no history of diabetes mellitus and normal baseline glucose values, 12 patients suffering from diabetes and no infection and 24 diabetic patients with severe sepsis were enrolled in the study. All septic patients were admitted in the Department of Medicine of Patras University Medical School during a period of 6 months, while diabetic non-septic patients were enrolled from the outpatient clinics of the Endocrinology Unit. RBC deformability was measured using a filtration method based on the initial flow rate conditions of blood samples suspension by using a Nuclepore membrane based Haemorheometer. Values are expressed as the Index of Rigidity (IR)and high IR values indicate low RBC deformability
Results: We observed no differences in severity, organ dysfunction and final outcome between diabetic and non-diabetic septic patients. Mean SAPS II score was 23.5% vs 26.8% in non-diabetic and diabetic septic patients accordingly. The mortality in the group of non-diabetic septic patients was 22.5% and in the group of septic diabetics 34.3%, while septic shock occurred in 15.0% and 20.8% accordingly. Patients with sepsis and patients with diabetes mellitus had significantly ($p < 0.01$)higher IR than healthy controls (13.9±2.86 and 12.26±2.28 vs 8.46±1.21, accordingly. Most significantly, diabetes mellitus and severe sepsis had an additive effect on RBC deformability, as IR in those patients was significantly higher than patients with diabetes and sepsis alone (17.72±6.31).
Conclusion: It seems that both diabetes mellitus and severe sepsis compromise the RBC deformability and increase the RBC rigidity index. Interestingly, when they co-exist, they seem to have additive effects. This supports the hypothesis that the presence of diabetes mellitus further affects the already compromised deformability of the red blood cells and this may contribute to the microcirculatory functional impairments in septic diabetic patients.

P1067 Alterations of innate and adaptive immune responses of patients with septic syndrome due to ventilator-associated pneumonia

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Objectives: Sepsis is the clinical expression of deregulated immune response. This study aims at investigating the expression of immune system components in septic patients with ventilator-associated pneumonia (VAP), the leading cause of morbidity and mortality among nosocomial infections in intensive care units.
Methods: Peripheral venous blood was sampled from 52 patients with sepsis, severe sepsis or septic shock (according to ACCP/SCCM 1992 criteria) within 24 hours of diagnosis. Twenty six suffered from VAP; 26 from other infections, namely pyelonephritis, bacteraemia and intraabdominal infection, all well-matched for severity, age and sex and they were used as controls. After lysis of red blood cells with ammonium chloride, white blood cells (WBCs) were stained by FITC-conjugated monoclonal antibodies for CD3, CD19, CD14, Annexin-V, and PE-conjugated antibodies for CD4, CD8, CD(16+56) and HLA-DR. Flow cytometry was used to determine subpopulations of mononuclear cells, apoptosis and HLA-DR expression.
Results: In patients with VAP, median expression of CD3/CD4, CD3/CD8, CD3/CD(16+56), CD(16+56) and CD19 was found in 35.01%, 21.29%, 4.02%, 8.91% and 8.89% of WBCs respectively. Moreover, expression of Annexin-V (marker of apoptosis) on monocytes, T-helper and T-cytotoxic lymphocytes was 15.39%, 4.90% and 2.98% respectively. CD(16+56) expression was positively correlated to both CD3/CD8 and CD14/HLA-DR (p 0.026 and 0.019 respectively). Median CD14/HLA-DR in patients with VAP was 56.88% and in controls 73.17% (p 0.046). Respective expression of CD3/CD(16+56) was 4.02% and 6.62% (p 0.025) and of CD(16+56) 8.92% and 18.07% (p 0.011).
Conclusions: In patients with VAP increased immunoparalysis is found as evidenced by lower CD14/HLA-DR expression. This is accompanied by decrease of T-LGL and NK cells and may explain increased mortality observed among patients with VAP. It may also constitute a future therapeutic target.

P1068 Triggering receptor expressed on myeloid cells (TREM-1) gene expression in critically ill patients

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Objectives: Triggering receptor expressed on myeloid cells (TREM-1) is a recently discovered receptor on monocytes and neutrophils. When stimulated, TREM-1 amplifies the toll-like receptor(TLR)-initiated response to invading pathogens allowing the secretion of pro-inflammatory chemokines and cytokines. Our aim was to estimate TREM-1 mRNA levels in critically ill patients.
Methods: Ten ml of heparinised venus blood were collected upon admission from 14 critically ill patients, five with systemic inflammatory response syndrome (SIRS) and nine with severe sepsis/septic shock (ACCP/SCCM criteria). Peripheral blood mononuclear cells were isolated. RNA was extracted after trizol and chloroform treatment and cDNA was synthesised. Expression of mTREM-1 was estimated by real time PCR against the expression of reference gene of beta-2-microglobulin. RNA isolated from PBMCs of healthy donor was applied, as control.
Results: Quantification of mRNA transcription levels using PFAFL equation showed that the levels of mRNA were higher in patients with SIRS (range 2.9×10^3–1.5×10^7, median 1×10^6 copies) compared to patients with severe sepsis (range 13.3–3×10^6, median 1×10^3 copies).
Conclusions: Although former results using flow cytometry analysis has showed that TREM-1 receptor is up regulated in sepsis, pronounced TREM-1 gene expression was found in SIRS compared to severe sepsis/septic shock. These results render the hypothesis that when

TREM-1 receptors reach a critical amount to amplifie the inflammatory response, internal signals stop further mTREM transcription.

P1069 Detection of superantigenic toxin Vbeta T-cell signatures during menstrual and non-menstrual staphylococcal toxic shock syndrome but not during *S. aureus* septic shock

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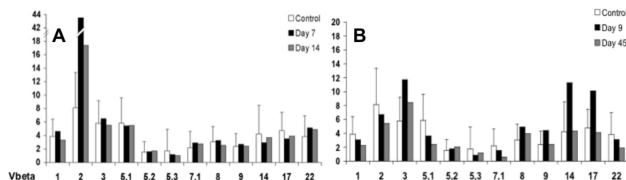
Objectives: To detect in vivo production of superantigenic toxins early after onset of staphylococcal toxic shock syndrome (TSS) and septic shock by identifying superantigenic toxin discriminant Vbeta signature (DVbS) in patient's peripheral blood mononuclear cells (PBMC).

Methods: Patients with *S. aureus* TSS or septic shock were prospectively enrolled in Lyon, France, during one year. The Vbeta repertoire of each patient was determined by flow cytometry at the onset of shock after purification (by Ficoll density gradient sedimentation) of PBMC labeled with anti-CD3 and 24 anti-Vbeta antibodies. The toxin gene content of all *S. aureus* isolates was determined by multiplex PCR. We determined which Vbeta subset was significantly expanded in the presence of each superantigenic toxin gene (i.e. the DVbS of a given superantigenic toxin) in vitro with PBMC from healthy donors incubated nine days with culture supernatant of each isolates.

Results: 14 patients were included, 5 with TSS (including 3 menstrual) and 9 septic shock. Patient with septic shock, in comparison with patients who had TSS, were older (61 vs 30 years), were lymphopenic (0.8 vs 2.5 G/L), had more frequently a medical history of immunosuppression (44% vs 0%) and had a higher mortality rate (56% vs 0%). In vitro, the DVbS were as follows: TSST-1: Vbeta 2; SEA: Vbeta 9, 22; SEB: Vbeta 3, 14, 17; SED: Vbeta 1, 8; egc: Vbeta 5.3, 7.1, 9, 23; and SElK: Vbeta 5.1. The DVbS of TSST-1 and SEB were detected in patients with menstrual and non-menstrual TSS (Figure, panel A and B, respectively), whereas no Vbeta signature was detected during septic shock (despite isolates produced superantigenic toxins in vitro).



Conclusion: Early analysis of the Vbeta T cell repertoire in patients with staphylococcal TSS: (i) is useful for detecting the superantigenic toxin DVbS; (ii) may help to confirm the diagnosis of TSS and hasten the administration of anti-toxin therapy. During septic shock, this approach failed to demonstrate superantigenic toxin involvement. However, a medical history of immunosuppression and lymphopenia due to apoptosis in this patient population may limit the expansion of targeted T cells.

P1070 Evaluation of serum TNF-alpha, IL-10, leptin, CRP levels as prognostic markers in patients with sepsis and septic shock

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Aim: The aim of this study was to determine the diagnostic value of serum TNF-alpha, IL-10, leptin, CRP levels in differentiating sepsis from septic shock and the prognostic value of these evels in predicting mortality or outcome in patients with sepsis and septic shock.

Material and Methods: Forty-sixth patients with sepsis (32 of sepsis and 14 of septic shock) and 50 healthy subjects were inluded in the study. Serum TNF-alpha, IL-10, leptin, CRP levels were compared between patients with sepsis and septic shock and healthy subjects and the prognostic value of these markers in predicting mortality were determined. For this aim, before treatment(1st day) and during the 3rd and 5th days of treatment serum TNF-alpha, IL-10, leptin, CRP levels were compared between patients with sepsis and septic shock and with those of healthy controls. For statistical evaluation, Mann-Whitney and Fisher's Exact tests were used.

Results: Serum CRP levels were significantly lower in patients with septic shock than sepsis before treatment. On the 3rd day of treatment, there was no difference in any parameter other than CRP levels. While serum CRP levels were found to be low in patients with sepsis on 3rd day, they were increased in patients with septic shock. On the 5th day of treatment, no difference was found in any parameter between patients with sepsis and septic shock.

In the patient group (both sepsis and septic shock), in the comparison between patients who survive and those who die, no difference was found in the parameters before treatment except for IL-10 levels, serum IL-10 levels being higher in the patients who died. On the 3rd day of treatment, serum TNF-alpha and CRP levels were different between surviving and dying patients. Serum TNF-alpha and CRP levels were higher in dying patients than those in surviving patients. On the 5th day of treatment, merely serum TNF-alpha levels were different between dying and surviving patients, values being higher in dying patients.

Conclusion: When the diagnostic value and prognostic values of these markers were evaluated, CRP levels were useful in discriminating between sepsis and septic shock before treatment and on the 3rd day of treatment, whilst IL-10 and TNF-alpha levels were useful in predicting mortality in patients with sepsis before treatment and on the 5th day of treatment respectively.

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P1071 Risk factors for meticillin-resistance among patients with *Staphylococcus aureus* sepsis

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Community-acquired infections caused by meticillin-resistant *Staphylococcus aureus* (MRSA) seem to be increasing. Characteristics permitting recognition of patients with such strains would aid infection control efforts and choice of empiric therapy pending culture and susceptibility. The aim of the study was to develop an objective mechanism to estimate the probability of meticillin resistance in a given patient with *Staphylococcus aureus* sepsis.

Material and Method: Retrospective review of medical records for all adults patients having at admittance in ICU Teaching Hospital of Infectious Diseases (Ian 2006-sept 2007) the diagnosis criteria for sepsis, according to ACCP/SCCM expert panel recommendation, and staphylococcal aetiology. Risk factors for the presence of meticillin resistance in *Staphylococcus aureus* isolates recovered from patients with staphylococcal sepsis were assessed.

Results: From 285 adult patients non-HIV with sepsis (27% nosocomial sepsis), we selected 50 cases (17.54%) with confirmed *Staphylococcus aureus* aetiology. Median age was 51.6 years, 26 males (52%). The MRSA rate was 30%. Of 7 characteristics included in the logistic regression (age, hospital onset, number of SIRS criteria, skin ulcers, respiratory involvement, shock, positive blood cultures), the only independent feature for MRSA was hospital onset ($p = 0.04$)

Conclusions: Through multivariate modeling techniques we confirm that nosocomiality is the most important determinant for MRSA and that patients with MRSA bacteraemia were not significantly older or more severe than those with meticillin-sensitive *Staphylococcus aureus* bacteraemia.

P1072 Recognising patients with sepsis in the emergency department before and after protocol implementation

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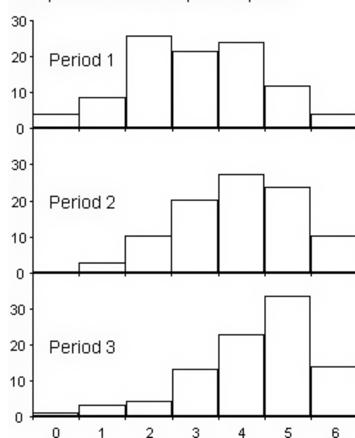
Objectives: The aim of the present study is to evaluate the effects of implementation of the Surviving Sepsis Campaign guideline in all septic patients in the Emergency Department (ED). Patients ⩾16 years

with sepsis in the ED at a 953 beds University Medical Centre in The Netherlands were included in the study.
Methods: Data were collected and analysed by means of a prospective cohort study from July 2006 to August 2007. The inclusion criteria were: (suspicion of) an infection AND presence of ⩾2 SIRS criteria extended with an altered consciousness, cold chills, SBP < 90 mmHg, MAP < 65 mmHg, hyperglycaemia in the absence of diabetes mellitus.
A multidisciplinary team developed the new sepsis guideline for nurses and doctors in the ED, which was introduced at the ED in November 2006.
The six major actions (6 items bundle) which were included in the sepsis guideline are: measure lactate ⩽6 hrs, take 2 blood cultures before start antibiotics, start antibiotics ⩽3 hrs, perform a chest X-ray, take urine for sediment and culture and hospitalise or discharge the patient ⩽3 hrs.
Results: 765 patients (451 men, 314 woman) with sepsis in the ED were included in the study. 159 patients before protocol implementation (period I), 447 patients after introduction of the protocol (period II) and 159 patients were included after presentation of the first results (period III). Compliance with the six major actions described in the methods (1 point earned per item) improved significantly ($p < 0.0001$) as illustrated in the figure. Hospital length of stay was median [25–75%range] 6 [2–12], 7 [3–12], 6 [3–11] days in period I, II and III, respectively. The hospital mortality rate was 6.3, 6.0 and 5.0% in period I, II and III, respectively (p=NS).
Conclusion: Implementation of a sepsis guideline resulted in a significant improvement of the compliance to the guideline. This improvement tended to be associated with a decrease in hospital mortality.
The management, diagnosis and treatment for patients with sepsis in the ED can be improved by implementation of a sepsis guideline.



P1073 Early pattern of IL-6, TNF-alpha, IL-12 and IFN-gamma levels in septic patients

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Objectives: Overcoming severe infections requires effective response involving both the innate and adaptive immunity. Priming and modulation of the immune response are driven by bacterial products and several endogenous mediators, such as cytokines. We analysed several proinflammatory cytokines in the early sepsis and studied their correlation with aetiology and severity.
Methods: In 24 septic patients we analysed serum levels of IL-6, TNF-a, IL-12 and IFN-g at day 1, 2, 3 and 7 upon admission. Patients (19 males/5 females) had the median age of 65.5. The primary infection had urinary (11), respiratory (11), dental (1) and cutaneous (1) origin. 14 healthy volunteers served as controls.
Results: At admission, median PCT was 10 ng/ml (0.5–10) and median SOFA score was 6 (range 1–11). 5 patients (17%) died during the first week. IL-12 and IFN-g were undetectable both in controls and septic patients at all time-points. Septic patients had median TNF-a concentration of 6 pg/ml at admission and decreasing at the following time-points, while in controls TNF-a was undetectable. In septic patients, IL-6 had a median level of 78 pg/ml at day 1 and decreased at 38 pg/ml, 9.15 pg/ml, 2.97 pg/ml at day 2, 3 and 7 respectively. Patients with severe sepsis (n = 38) had the highest IL-6 levels, reaching a median of 102.2 pg/ml, patients with sepsis had a median value of 61.2 pg/ml while IL-6 in controls was 1.78 pg/ml. TNF-a showed similar levels and kinetics between urinary and respiratory sepsis, yet in respiratory sepsis IL-6 increased more prominently. Gram-positive sepsis led to higher amounts of circulating TNF-a and IL-6, reaching 23.6 pg/ml and 130 pg/ml respectively while in Gram-negative sepsis, median levels of TNF-a and IL-6 were 6.6 pg/ml and 60 pg/ml respectively. Two non-survivors had very high IL-6 at day 1 (over 1000 pg/ml) that did not follow the descendent trend of IL-6 levels at next time-points.
Conclusions: During early sepsis, a rapid inflammatory response is mounted and driven by the innate immunity, yet excessive response may prove detrimental. Since we did not detect circulating levels of IL-12 and IFN-g, it appears that sepsis does not to elicit an adaptive immune response in its early phases. The low levels of TNF-a are maybe due to the short half-life of the molecule. IL-6 release might be influenced by the primary site of infection and aetiology, and it could serve as a potential routine, reliable parameter in monitoring septic patients.

P1074 Statins for infection and sepsis – A systematic review of the clinical evidence

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Objective: Statins are currently used for hyperlipidaemia control and considered useful in protecting from cardiovascular events. In addition, there is increasing use of evidence for the potential use of statins in treating infections.
Methods: We performed a systematic review of the literature that compared the outcome between statin and non-statin users among patients suffering from sepsis or other infections (bacteraemia, pneumonia, ICU infection, viral infection and other bacterial infection). The relevant studies were identified from searches of the PubMed and the Cochrane Library Databases.
Results: Twenty studies were identified out of which 9 examined the use of statins in patients with sepsis, bacteraemia, or multi-organ dysfunction syndrome, 4 community acquired pneumonia (CAP), 1 ICU infections, 2 other bacterial infections, and 4 viral infections. In total, 11 studies had data regarding mortality as the main outcome: 8 showed decreased mortality in statins users (3 of them reported on patients with bacteraemia), 2 showed no difference in mortality and 1 reported an increased mortality in patients who received statins. Seven studies examined the risk of sepsis as the main outcome; 6 of these studies showed a decreased risk of sepsis in patients receiving statins, while one study found no difference.
Conclusion: The majority of the studies suggest that statins may have a positive role in the treatment of patients with sepsis and infection. However, the reviewed studies have several methodological limitations. Conclusions regarding this important clinical question should wait for the results of ongoing relevant randomised controlled trials.

P1075 Anti-platelet activating factor effects of antibiotics: a new insight into therapy of sepsis?

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Objectives: There are data supporting the role of inflammatory mediators, such as anti-platelet activating factor (PAF), in sepsis. These molecules can act in synergy with infectious agents to initiate and propagate the disease process. In animal models, beneficial effects have been observed as a result of treatment with various antagonists of PAF;

however the precise role of PAF as mediator of the diffuse inflammatory state characteristic of sepsis remains to be determined.
Methods: In order to examine the possible interactions between PAF and antibiotic treatment, we studied the effect of different compounds against PAF biological activity. We assessed the in vitro inhibitory effect of various compounds against PAF-induced aggregation on washed rabbit platelets by evaluating the IC50 of each drug. We also studied the in vitro effect of these drugs against rabbit plasma PAF-AH (the main degradating enzyme of PAF) and PAF-CPT (the main de novo biosynthetic enzyme of PAF) of washed rabbit leukocytes.
Results: Clarithromycin, azithromycin, linezolid, amikacin, netilmicin and piperacillin/tazobactam exhibited IC50: 0.06, 0.1, 0.29, 0.73, 0.74 and 4.92 μg/μL respectively. Concentrations equivalent to their IC50 values against PAF activity, inhibited in vitro the PAF-CPT activity of washed rabbit leukocytes. Furthermore, clarithromycin and azithromycin in concentrations of a log10 higher than their IC50 values against PAF, increased in vitro the rabbit plasma PAF-AH (table 1).

Drug	μg/μL	% Inhibition of PAF-CPT	% Increase of PAF-AH
Clarithromycin	0.04	59.09	
	0.21		34.73
Azithromycin	0.16	34.85	
	1.56		40.12
Piperacillin/tazobactam	2.81	62.94	
Amikacin	0.31	60.26	
Netilmicin	0.5	47.68	
Linezolid	0.25	51.22	

Conclusion: Many antibiotics exhibit high inhibitory effect against PAF-induced washed rabbit platelet aggregation and its main de novo biosynthetic enzyme PAF-CPT while only clarithromycin and azithromycin seem to activate plasma PAF-AH. The promising in vitro results need to be further studied and confirmed by in vivo tests, in order to optimise the efficacy of antibiotic treatment in sepsis.

P1076 The natriuretic peptide (BNP) as a marker of sepsis

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Objective: The discovery of a reliable marker for sepsis is of great significance for the on time management and survival of septic patients. The aim of the study is the evaluation of the reliability of the Natriuretic peptide (BNP) as a marker of sepsis in comparison to C Reactive protein.
Methods: We studied 58 patients of the ICU in a year period. They were separated into 2 groups. Group A with 8 non septic patients and Group B with 50 patients suffering from great sepsis and septic shock. Blood samples were taken from all patients at their admittance and at days 2, 3, 4, 6, 8 and 10. The levels of BNP were analysed with microsomatic immunoenzyme method (AXSYM – ABBOT) (Normal value <100 pg/ml) and the levels of CRP with nefelometry (Normal value <5 mg/ l).
Patients with heart failure were excluded from the study.
Results: The mean value of BNP in Group A was 24.2 pg/ml, while the mean value of BNP in Group B was 971.2 pg/ml. The difference is statistically important.
The mean value of CRP in Group A was 103.3 mg/l, while in Group B it was 107.062 mg/l. The difference has no statistical importance.
Conclusion: The evaluation of the results seem to drive to the conclusion that the levels of BNP are reliably increased in patients with great sepsis and septic shock and may be a valuable diagnostic tool.

P1077 Diagnostic value of the soluble triggering receptor expressed on myeloid cells-1 in critically ill patients with suspected sepsis

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Objective: To evaluate the diagnostic and prognosis value of plasma levels of the soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) in critically ill patients admitted with clinical suspicion of sepsis.
Methods: All consecutive adult patients, who were newly hospitalised in the surgical intensive care unit (SICU) from January to October 2006, were prospectively enrolled in this study if they had clinical suspected infection and fulfilled at least 2 criteria of the systemic inflammatory response syndrome (SIRS). Patients were not enrolled if they were older than 80-year-old, were immunocompromised, died or were discharged within 12 hours after admission. Within 12 hours after enrollment, the levels of plasma sTREM-1 and CRP, TNF-Alfa, IL-6, procalcitonin (PCT) were measured, and the microbiological cultures were performed. Base on the results of cultures and the decision of intensivists, all patients were classified into different groups as SIRS (no infection), sepsis, severe sepsis, or septic shock. The levels of plasma sTREM-1 and CRP, TNF-Alfa, IL-6, and PCT in different groups were compared.
Results: There was a significant difference of median plasma sTREM-1 levels at admission between different groups (68.8 pg/ml in SIRS group, 69.7 pg/ml in sepsis group, 106.2 pg/ml in severe sepsis group, 373.5 pg/ml in septic shock group, $P < 0.001$). The capacity of Plasma sTREM-1 to differentiate the presence of sepsis or septic shock was assessed with a receiver-operating-characteristic curve (ROC) analysis. The area under the ROC when Plasma sTREM-1 was used to differentiate the presence of sepsis was 0.82 (95% CI, 0.70–0.94, $P < 0.001$). The area under the ROC when Plasma sTREM-1 was used to differentiate the presence of septic shock was 0.96 (95% CI, 0.90–1.0, $P < 0.001$). Only sTREM-1 was found to be an independent predictor of the prognosis with sepsis. The area under the ROC was 0.87(95% CI, 0.77–0.97, $P < 0.001$), when Plasma sTREM-1 was used to prognoses the presence of death in sepsis patients.
Conclusion: There were higher plasma sTREM-1 levels in critically ill patients with sepsis syndromes. TREM-1 was an effective indicator of diagnosis of sepsis and septic shock, and also an effective prognosis risk predictor of sepsis in critically ill patients.

P1078 Comparison of procalcitonin with interleukin 8, leucocyte count and rate of immature to total neutrophils for the early diagnosis of severe bacterial pneumonia and sepsis in pre-term infants

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Objective: To evaluate procalcitonin (PCT) as a test for early diagnosis of bacterial pneumonia and sepsis in preterm infants and to compare the results of PCT with those of interleukin 8 (IL-8), leucocyte count, rate of immature to total neutrophils (I/T).
Subjects and Methods: Serum PCT (BRAMS PCT-Q™) was prospectively measured along with IL-8 (ELISA), leucocyte count in 82 preterm infants, 26–36 wk of gestation. Preterm infants were classified to two groups according to their infections status: (1) 48 preterm infants with severe bacterial pneumonia and sepsis (positive blood cultures), (2) 34 noninfected preterm infants wiht respiratory distress syndrome (RDS). PCT, IL-8, leucocyte count, I/T were analysed for sensitivity, specificity and positive and negative predictive values.
Results: At a cut off value of 2ng/ml, the sensitivity of PCT was 92% and specificity was 97%. The positive and negative predictive values of PCT in the diagnosis equalled 97.7% and 89.1%, respectively. Sensitivities for IL-8 (⩾70ng/ml), leucocyte count (⩾15·109/l) and I/T (⩾0.2) was 50%, 66%, 35% and specificities – 94%, 71%, 88%, respectively.
Conclusion: PCT was the most specificity and sensitive diagnostic parameter in the diagnosis of severe bacterial pneumonia and sepsis.

A PCT concentration ⩾2ng/ml might be useful in differentiating severe bacterial pneumonia and sepsis in preterm infants.

P1079 Comparison of neutrophil volume distribution width with C-reactive protein and procalcitonin for diagnosis of culture-proven sepsis

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Objectives: Rapid diagnosis of bloodstream infection is problematic due to time-consuming blood culture. Current biomarkers in diagnosis of sepsis either lack of sensitivity and specificity or are expensive. Neutrophil volume distribution width (NDW) which is generated on the hematology analyser when measuring complete blood count has been demonstrated by Chaves et al (2005) as a potential predictor of sepsis in a fast and cost-effective manner. However, its clinical usefulness remains un-established. In this study, NDW values were compared with plasma concentrations of C-reactive protein (CRP) and procalcitonin (PCT) for diagnosis of culture-proven sepsis.
Methods: Within 12 hours of blood culture submission, NDW and concentrations of CRP and PCT of peripheral blood samples were retrospectively reviewed or analysed. After exclusion of contaminated samples in blood culture, only laboratory data obtained from patient samples of positive bacterial blood culture were recruited for comparison with those of negative culture. Among them, NDW, CRP and PCT were measured using VCS technology of LH750 hematology analyser (Beckman Coulter), immunoturbidometry (COBAS INTEGRA 800, Roche Diagnostics) and enzyme-linked fluorescent assay (VIDAS BRAHMS PCT, bioMerieux), respectively. Positive blood culture was used as the gold standard for diagnosis of sepsis. Receiver operating characteristic (ROC) curve was constructed and area under ROC curves (AUC) was determined for evaluation of diagnostic power.
Results: Laboratory data of 194 unrelated patients (age of range, 0–101 years), were collected. Of them, 60 and 134 had positive and negative bacterial culture, respectively. To predict bacteraemia, NDW had a similar AUC (0.78 [0.70–0.85], $p < 0.001$) as PCT (0.80, [0.73–0.87], $p < 0.001$) but CRP had a lower AUC (0.64 [0.56–0.72], $p = 0.002$). The optimal sensitivity and specificity at an appropriate cutoff value were 0.72 and 0.73 at 24.5 for NDW, 0.75 and 0.70 at 0.6 ng/mL for PCT, and 0.59 and 0.56 at 63.3 mg/L for CRP, respectively.
Conclusion: Our preliminary study had demonstrated that NDW had a superior diagnostic accuracy in bacteraemia than CRP as PCT in prediction of bacteraemia. This finding might imply a potential role of NDW for laboratory prediction of culture-proven sepsis in a fast and cost-effective manner.

P1080 Procalcitonin as a diagnostic and prognostic marker in chronically-critically ill patients

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Objective: To investigate Procalcitonin (PCT) levels as prognostic markers of infection and to correlate maximum as well as every other day changes of PCT levels to mortality of chronically critically ill patients.
Methods: This is study including all patients hospitalised for more than 10 days in ICU. Blood samples for PCT, WBC, and CRP were drawn every other day. Patients were classified to one of the following groups according to ACCPM 1994 criteria 0: no infection, 1: localised infection and sepsis, 2: severe sepsis and septic shock and SOFA score was recorded. PCT concentrations were measured by an automated immunofluorescent assay (PCT KRYPTOR) PCT values were categorised as alert and non alert values using x-bar test. Maximum PCT (PCT max) levels and number of days with PCT higher than the alert value were compared between survivors and non-survivors. All PCT levels were correlated with CRP, WBC and SOFA score with the Spearman test and compared among the various groups of severity of infection by one-way ANOVA. Categorical variables were compared using the chi-square test and continuous variables with non-parametric Mann–Whitney U test.
Results: PCT plasma concentrations were measured in a total of 169 samples collected from 11 patients (8 male and 3 female) followed up for a mean of 34.5 days (range 14 to 67) until discharge or death. The alert value in this population was calculated as 0.88 ng/ml. Mean PCT (±SD) concentration of patients categorised as having no infection, sepsis and severe sepsis–septic shock were 0.47 (±0.40), 1.86 (±3.86) and 4.82 (±7.58) respectively ($p < 0.0001$). There was a definite but weak correlation between PCT concentrations, CRP (Spearman's rho 0.21, $p < 0.007$) and SOFA score (Spearman's rho 0.3, $p < 0.0001$) but not with WBC. PCT max was 4.33 in survivors and 12.98 in non-survivors (p NS). Mean total days with PCT higher than alert value were 2.44 and 5.66 respectively (p NS)
Conclusions: Mean PCT plasma concentrations correlated to the severity of the infection. There was a weak correlation among PCT, CRP and SOFA score and a trend to higher mean PCT values and to more mean total days above the alert value in non-survivors than survivors.

P1081 Evaluation of F-18-fluorodeoxyglucose positron emission combined with computer tomography (FDG-PET-CT-scan) in critically ill patients suspected of Infection

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Objectives: During their stay at the intensive care unit (ICU), many critically ill patients are suspected of a focal infectious process. Timely identification and localisation of infection is critical in appropriate treatment of these patients. FDG-PET has shown to be valuable in the evaluation of fever of unknown origin and suspected focal infectious diseases in non-ICU patients, but no studies have been performed in critically ill patients. In this study, the value of FDG-PET-CT-scanning in diagnosing focal infection was studied in ICU patients.
Methods: Between October 2005 and June 2007 all FDG-PET-CT scans ordered in ICU patients for evaluation of persisting fever or suspected focal infection were retrospectively analysed. The final diagnosis was used for comparison with FDG-PET-CT results. A diagnosis was never based on the FDG-PET-CT results alone.
Results: 29 FDG-PET-CT scans were performed in 28 ICU patients (including 4 children, median age 58 years). In fifteen patients FDG-PET was performed because of persisting fever (median duration of fever 17 days) and in 15 patients because of suspected focal infection. Seventeen patients were eventually diagnosed with an infection, one patient with malignant lymphoma, and in 12 cases no diagnosis was reached. The PET-CT scan was performed after a mean ICU-stay of 22 ±2.8 days. No complications occurred during the transportation and scanning procedure. Sixteen FDG-PET-CT scans were classified true positive and were considered to be contributory to the final diagnosis. Three PET-CT scans were considered false positive, in one case leading to additional diagnostic procedures. Additionally, ten true negative and no false negative scans were found. Thus, 84% of the abnormal FDG-PET scans were clinically helpful and FDG-PET contributed to the ultimate diagnosis in 55% of all patients. FDG-PET was considered helpful in 94% of all patients with a final diagnosis.
Conclusion: FDG-PET-CT scanning is of additional value in the evaluation of persisting fever and suspected focal infection in critically ill patients. A normal FDG-PET-CT scan also ruled out a focal infection with a very high negative predictive value.

P1082 Is venous access port inner surface colonisation related with port obstruction?

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Objectives: Venous acces ports (VAP) are used in patients receiving long parenteral therapy. However, use of these devices is associated with infectious and mechanical complications. One of the most

common causes of mechanical failure is catheter lumen occlusion. This complication may lead to the removal of the port. Although precipitation of drugs and blood clots can obstruct the device, we propose here that colonisation of the port inner surface may be related to port obstruction. The aim of this study was to analyse if port colonisation and obstruction were associated in a significative way.

Methods: We performed a 27 months prospective study on all the removed VAPs of our hospital. We analysed reasons for removal and we cultured all the removed ports (catheter tip and septum culture after a vortexing-sonication procedure). We compared colonisation ratios on the group of patients whose VAPs were removed because of the obstruction (group 1) and the group of patients whose VAPs were removed because of end of use or because of a mechanical complication other than obstruction (group 2). Patients with port-related infections were excluded. The cut-off to consider the ports to be colonised was >1000 cfu/ml for both cultures (catheter and septum). Differences were statistically analysed using the chi-square test.

Results: From August 2005 to October 2007, 240 VAPs were removed from 240 patients. Ports were removed due to end of use in 187 (77.9%) cases, 34 (14.2%) ports were removed because of infectious complications and 19 (7.9%) because of mechanical complications. Seven of the ports were removed due to obstruction (group 1) and 197 because end of use (group 2). Colonisation rates were 3/7 (42.9%) and 16/197 (8.1%) in groups 1 and 2 respectively. Differences were statistically significative with a p value of <0.019. Coagulase-negative staphylococci were the most frequent isolated microorganisms, followed by *Candida* species.

Conclusions: Data from this study suggest that port colonisation may play a role in the genesis of port obstruction. Antibiotic lock therapy might be useful in this scenario to try to avoid removal because of obstruction. However, more prospective studies are warranted to address this important issue.

Clinical epidemiology of bloodstream infections and occupational blood exposure

P1083 Incidence of central venous catheter-related infection in a community hospital

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Introduction: Catheter-related infections (CRI) are a main cause of nosocomial bloodstream infections and are related to important morbidity and mortality. In the Spanish Nosocomial Infection Study (EPINE) the prevalence of catheter-related bloodstream infection (CRBI) is between 2.5 and 3.4 episodes per 1000 hospitalised patients.

Objective: To asses the incidence of central venous catheter related bloodstream infections (CRBI) in a community hospital.

Methods: Prospective daily follow-up of all central venous catheters (CVC) inserted in the Hospital de Mataro during a period of 9 months. Main patient's demographic data and catheters characteristics were recorded. All catheters were inserted using full sterile barrier methods. Hubs were closed with disinfectable needleless mechanical valve connectors. Catheters removed by suspicion of infection were semiquantitatively cultured using the roll plate method. Two blood cultures were also performed. According to clinical and microbiological criteria, catheters were classified as: not infection-related, entry-site infection, colonised catheter, and CRBI.

Results: During 9 months 597 catheters were placed in 480 patients, 282 males (58%). Total catheterisation days were 4886. Mean catheterisation days were 8.19 (1–58). Catheters were inserted in the emergency room in 35 cases (5.8%), 151 in surgical theater (25.2%), 45 in others units (7.23%) and 365 in ICU (61%) where a trained and specialised nurse team exists. The subclavian access were used in 194 catheters (32.49%), jugular in 124 (20.7%), brachial in 245 (41%) and femoral in 30 (5.02%). Catheters were removed by end of treatment in 419 cases (70.18%), dysfunction in 42 cases (7.03%), and by fever or suspicion of infection in 40 patients (6.7%). Seven episodes of CRBI were registered, all of them caused by *S. epidermidis*, which results in an incidence rate of 1.43 episodes/catheterisation days. No CRBI was observed during this period in the ICU, resulting in an incidence rate of 0 in ICU and 2.25 episodes/1000 catheterisation days in conventional hospitalisation units.

Conclusion: The incidence of CRBI in our institution is in the accepted normal range. We observe relevant differences in CRBI's rates between conventional hospitalisation units and ICU where a trained and specialised nurse team exists. This suggests the importance of catheter manipulation in the genesis and prevention of CRBI.

P1084 Use of disinfectable needleless mechanical valve connectors and catheter-related bloodstream infection rates in a polyvalent intensive care unit

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Introduction: Disinfectable needleless mechanical valve connectors (DNMVC) have been introduced to avoid needlestick injuries in healthcare workers but they are accompanied by concerns about the possibility of an increase in the risk of catheter-related bloodstream infections (CRBI) associated with their incorrect use. Use of bundles can facilitate the adherence to the handling recommendations.

Objective: To observe the rates of CRBI after the development of a handling bundle in a polyvalent intensive care unit (ICU) where a DNMVC (Smartsite) are used to close the intravenous catheter hubs.

Method: We used a 5 recommendations bundle (nurse implication in the epidemiological surveillance, selection of the safest place of insertion, understand insertion as a surgical procedure, maintain the endoluminal sterility during infusion and avoid unnecessary insertions, manipulations or removals). Every three months data were reported to the nurse shift that was responsible of surveillance and diffusion of the results to the rest of the nurse shifts.

Results: From January to September 2007, a total of 510 catheters were inserted in 229 critically ill patients during more than 24 hours, 273 of they were central venous catheters and 237 arterial lines. The mean length of insertion was 6.3±6.0 days, and it supposes 3227 days of risk. We don't observe any CRBI and 2 primary bacteraemia (0 and 0.62 episodes per 1000 days of risk respectively).

Conclusions: Rates of CRBI and primary bacteraemia were minimal during the 9 months that the observational study was performed (0 and 0.62 episodes per 1000 days of risk). Inclusion of nurses as active part of the epidemiological surveillance can help the implementation of simple recommendations. We can not associate the use of disinfectable needleless mechanical valve connectors with increased rates of catheter-related bloodstream infections.

P1085 Catheter-related bloodstream infection in patients on total parenteral nutrition: 10-year surveillance data in a university teaching hospital in Dublin, Ireland

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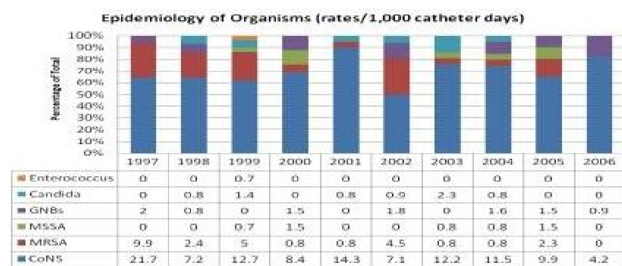
Objectives: Total parenteral nutrition (TPN) is a well-recognised risk factor for catheter-related bloodstream infection (CR-BSI). However, there is limited data in the literature specifically addressing CR-BSI in patients on TPN. Surveillance of CR-BSIs in patients on TPN has been ongoing in the Mater Misericordiae University Hospital (MMUH), a 525-tertiary referral hospital in Dublin for over 10 years. In 1997, a TPN surveillance Clinical Nurse Manager (CNM) was appointed. Protocols re insertion of central venous catheters (CVCs) specifically addressing location of insertion and skin preparation were introduced in 2002. Quarterly TPN committee meetings along with frequent educational sessions were introduced. Our aim was to demonstrate the epidemiology of the organisms causing CR-BSIs in this subset of patients and the impact of the above measures both on the incidence of CR-BSIs and the duration that the CVC remains in situ before infection occurs.

Methods: Data on all patients prescribed TPN has been collected prospectively. Individual cases were reviewed at quarterly meetings over this time period and cases were assigned to appropriate categories as per CDC definitions on CR-BSIs. All CVCs were non-antibiotic impregnated. The data was analysed only for non-tunnelled CVCs.
Results: 194 CR-BSIs in 1,197 patients (total of 12,296 catheter days) on TPN were recorded in the 10-year time period. Staphylococci accounted for over 80% of infections each year with few *Candida* spp. or Gram-negative infections (fig 1). A reduction in the overall incidence of CR-BSI (33.6 to 5.1/1,000 catheter days) and an increase in the duration that the catheter is in situ before infection occurs (mean 4.8 to 8.2 days) was noted.
Conclusions: The high proportion of CR-BSIs in patients on TPN due to staphylococci has important implications for treatment. Glycopeptide therapy alone may be sufficient empirical therapy in this subset of patients unless severely septic. Appointment of the surveillance CNM allied with auditing, feedback, education and insertion protocols led to positive effects on the incidence of CVC infection and catheter duration before infection.



	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006
Enterococcus	0	0	0.7	0	0	0	0	0	0	0
Candida	0	0.8	1.4	0	0.8	0.9	2.3	0.8	0	0
GNBs	2	0.8	0	1.5	0	1.8	0	1.6	1.5	0.9
MSSA	0	0	0.7	1.5	0	0	0.8	0.8	1.5	0
MRSA	9.9	2.4	5	0.8	0.8	4.5	0.8	0.8	2.3	0
CoNS	21.7	7.2	12.7	8.4	14.3	7.1	12.2	11.5	9.9	4.2

Epidemiology of Organisms (rates/1,000 catheter days)

P1086 Association of platelet parameters and Gram-negative bacteraemias: a case control study

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Background and Objective: Bacteraemia and sepsis, as uncontrolled generalised inflammatory responses, involve thrombopoiesis and haemostatic mechanisms. Mean platelet volume (MPV), platelet distribution width (PDW), their correlations with platelet count and inflammation markers and their clinical significance have not been studied in bacteraemias in depth. The aim of this study was 1) to determine whether newly diagnosed patients with Gram(–) bacteraemia, in comparison to their controls, would be characterised by ineffective thrombopoiesis, as reflected by MPV and PDW and 2) to explore their correlation with inflammatory markers and their significance in the course of bacteraemia.
Material and Methods: We have evaluated 22 incident cases of Gram-negative bacteraemia (13 men and 9 women, 23–82 years) and an equal number of hospital controls with minor non-infectious and non-neoplastic conditions, individually matched for age (±5 years) and gender and month of diagnosis. To assess thrombopoiesis and inflammation, we have determined platelet count, MPV, PDW and leucocyte count using Sysmex SE-9000 blood analyser. CRP levels were determined using immunonephelometry (Dade-Behring, Germany). Statistical analysis of data was performed with SAS® version 9 software.
Results: Significantly low mean values of MPV and PDW, together with significantly high mean values of platelet count, leucocyte count and CRP were shown in patients with Gram(–) bacteraemia in comparison to their controls ($p < 0.001$). There was not any statistical significant difference in platelet count, MPV, PDW and CRP levels ($p > 0.05$) in patients with Gram(–) bacteraemia based upon the number of leucocytes (those with leucopenia n = 3 and those with leucocytosis n = 37). There was a significant inverse relationship between MPV (and PDW) and platelet count in patients with bacteraemia (r=-0.78; $p < 0.001$) as well as a significant inverse relationship between MPV and CRP (r=-0.52; $p = 0.01$). Adjusting for age, gender and CRP levels, MPV (or PDW) is a significant predictor of bacteraemia. Patients with Gram(–) bacteraemia who responded favorably to antibiotic therapy presented normal platelet parameters after ten days of treatment.
Conclusion: Platelet parameters have been shown to provide an accessible and clinical useful index for estimating the impairment of thrombopoiesis and the degree of inflammation in patients with Gram-negative bacteraemia as well as for predicting favorable response to antibiotics.

P1087 Incidence and risk factors of recurrent bacteraemia due to *Escherichia coli*

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Objectives: *E. coli* recurrent bacteraemia (RB), defined as relapse or reinfection, is a relevant but not well known clinical process. The aim of this study was to determine the frequency of RB and risk factors for relapse (isolates with the same pulsed-field gel electrophoresis pattern [PFGE]) or reinfection (different PFGE) after a first episode of *E. coli* bacteraemia.
Methods: This is a retrospective study conducted in a tertiary Hospital. RB was defined as a new episode of bacteraemia after at least 1 month since a prior episode sucessfully treated. Patients with RB during 2001–2005 were included. The control group included 41 patients with a single episode of bacteraemia and a year of follow-up. A systematic review of clinical data was performed. Blood isolates were analysed by PFGE and the phylogenetic background studied. We compared demographic, clinical and microbiological variables between relapse or reinfection and control group.
Results: 945 patients had *E. coli* bacteraemia, 32 (3.4%) had RB. We studied blood isolates from 28 patients (4 excluded) with 58 clinical episodes. According to PFGE analysis, 13 patients (46.4%) suffered relapse and 15 (53.6%) reinfection. Relapse occurred earlier than reinfection (median, 56 versus 67 days, $p = 0.02$), and it was caused by strains more resistant to antibiotics. In the comparison with no RB, multivariate analysis showed that patients with relapse were more likely to be younger than 60 years (OR:17.5; $p = 0.05$), admitted in the nefrology unit (OR:246.9; $p = 0.012$), to have received antibiotics previously (OR:15.6; $p = 0.045$) and to be infected with ciprofloxacin resistant strains (OR: 97.0; $p = 0.008$). On the other hand, patients with reinfection were associated with prior hospital admissions (OR: 21.6; $p = 0.027$), being admitted in haematology/oncology units (OR: 19.8; $p = 0.037$), having a central intravascular catheters (OR:60.9; $p = 0.008$), and having a biliary/gastrointestinal source of infection (OR: 20.9; $p = 0.039$). The distribution of phylogenetic groups in relapses was: group B1 38.5%, D 30.8%, B2 23.1%, and A 7.7% and in reinfections was: group B1 33.3%, B2 25.9%, D 18.5%, and A 22.2%.
Conclusions: We have determined the frequency of recurrent *Escherichia coli* bacteraemia in our hospital and delineated some risk factors for relapse and reinfection. Appropriate management for the prevention of further recurrences may need more research on treatment strategies and virulence factors of *E. coli* strains

P1088 Invasive *Staphylococcus aureus* Infections Cohort (INSTINCT)

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Introduction: Nosocomial bloodstream infections (BSIs) are important causes of morbidity and *S. aureus* is one of the most important nosocomial pathogens.
Methods: INSTINCT was established to prospectively monitor clinical features of *S. aureus* BSI (SAB) in Germany using a web-based database. Data are collected by infectious diseases physicians aided by specially trained medical personnel to monitor risk factors, predisposing conditions, portal of entry, clinical course, diagnostic and therapeutic procedures as well as outcome up to one year after SAB.

Results: Between January 2005 and September 2007, 468 cases of SAB were detected in the 3 participating university hospitals. Patients had a mean age of 61 years and 33% were female. Length of stay averaged 33 days. Underlying conditions most often included cardiovascular (35%), or renal (27%) disorders and diabetes mellitus (27%). SAB were classified as nosocomial in 54% of patients another 26% were healthcare-associated. Primary SAB was seen in 61% of cases, of which 28% were catheter-related. Secondary SAB most often originated from skin and soft tissue infections (7%) or surgical wound infections (5%). 18% of *S. aureus* isolates were meticillin-resistant. Severe Sepsis and septic shock at onset of SAB were seen in 4.5% and 2.5% of patients, respectively, secondary complications were reported in 14% of patients, most commonly endocarditis, osteomyelitis or spondylodiscitis. The mean duration of *S. aureus* bacteraemia was 3.5 days. Patients received a mean of 16±13 days of appropriate anti-staphylococcal therapy. Adherence to ID consult recommendations such as TEE (recommended in 50%, followed in 34%) and follow-up blood-cultures (61%; 48%) varied significantly between centres. The crude 7-day-mortality was 15%, in-hospital mortality was 23%.

Conclusion: INSTINCT currently represents one of the most detailed prospective SAB studies. Data from the first years underscore the importance of SAB and confirm the feasibility of this very detailed monitoring design.

P1089 Primary bloodstream infections and catheter-related bloodstream infections in critically ill patients: impact on length of stay and mortality

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Objective: Previous studies have suggested that catheter-related bloodstream infections (CRBSIs) are associated with lower attributable mortality than other types of BSI. The aim of our study was to compare the impact of primary BSIs (PBSIs) and CRBSIs on length of stay (LOS) and mortality in intensive care unit (ICU) patients.

Patients and Methods: All patients admitted in our 8-bed multidisciplinary ICU were included in the study. Data recorded were: gender, age, admission category (medical, emergent surgical, elective surgical, trauma), Acute Physiology and Health II Evaluation (APACHE II) score at admission in the ICU. All patients were prospectively followed and the occurrence of the first BSI episode in the ICU was recorded. Length of stay (LOS) in the ICU, total number of BSI episodes in the ICU and ICU outcome were recorded. Primary BSI (PBSI) and catheter-related BSI (CRBSI) episodes defined according to standard criteria were analysed. Attributable mortality for PBSI and CRBSI was estimated and further investigated with logistic regression models adjusted for potential confounders. Statistical significance level was set at $p < 0.05$.

Results: Three hundred eighty-four consecutive patients (273 males and 111 females) were included in the study. Age (mean±SD) was 52.9±19.4 years, APACHE II score 18.2±6.4, LOS [median (interquartile range): 16 (9–32) days. In 76 patients (19.8%) the first BSI episode was PBSI and in 41 (10.7%) CRBSI. Overall mortality was 16.4%. Mortality in patients without BSI was 12.0%, in patients with PBSI 27.6% (attributable mortality 15.6%, $p = 0.001$) and in patients with CRBSI 19.5% (attributable mortality 7.5%, $p = 0.189$). LOS was significantly shorter in patients without BSI compared with either PBSI or CRBSI group (14.6±11.0 days vs. 39.0±26.4 days in the PBSI group and 39.8±20.2 days in the CRBSI group, $p < 0.001$). In a logistic regression model adjusted for age, gender, APACHE II score, admission category and total number of BSI episodes in the ICU, PBSI was significantly associated with increased probability of death in the ICU compared with patients without BSI (odds ratio=3.4, 95% confidence interval 1.2–10.7, $p = 0.033$) while CRBSI had no significant effect on mortality.

Conclusions: In our patient sample, PBSI increased 3.4-fold the probability of death in the ICU compared with patients without BSI. The estimated attributable mortality for PBSI was 15.6%. CRBSI did not significantly influence mortality.

P1090 The association between mucosal colonisation with opportunistic pathogens and bloodstream infections in the two neonatal intensive care units in Estonia

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Objective We aimed to study mucosal colonisation of neonates with potentially pathogenic microorganisms in order to find out whether and how this is associated with blood stream infections (BSI).

Methods All newborns admitted to both Estonian NICUs within first 72 h of life and needing empiric antibacterial therapy (ampicillin or penicillin in combination with gentamicin) for early onset neonatal sepsis were prospectively enrolled. The nasopharyngeal and rectal swabs were collected on admission and thereafter twice weekly until D60 or discharge. Samples were plated on blood, McConkey and Saboraud agar. Blood cultures were drawn when clinically indicated. The final identification of species was conducted with BACTEC 9240, VITEK 2 and API 20E systems. MRSA was identified by PCR for nuc and mecA genes. The genetic relatedness between mucosal and bacteraemic strains of MRSA and *K. pneumoniae* was analysed by pulsed fieled gel electrophoresis (PFGE).

Results A total of 200 pt (100 with BW <1500g; median NICU stay 6.8 days) were enrolled from Aug 2006 until July 2007. The most common colonising Gram-negative microorganisms were *K. pneumoniae* (23%), *E. coli* (15%), E.cloaceae (15%), *K. oxytoca* (14%) & *A. baumannii* (13%) with the median documented duration of colonisation of 8 days for *Klebsiella* spp. and 5 days for the remaining organisms. Of 54 BSI infections 45 were nosocomial origin (58% caused by G-positive, 40% by G-negative and 2% by *Candida* spp.). The risk of BSI was greater during colonisation by G-negative than by G-positive bacteria (15/21 vs 13/31, respectively [OR 3.5; 95% CI 1.1–11.3]). The rate of infection per 100 days of colonisation was the highest for MRSA (4.6) followed by 1.4; 1.2; 0.9; 0.7; & 0.7 for *A. baumannii*, *E. cloacae*, *E. coli*, *K. pneumoniae* & *Serratia* spp., respectively. The lowest rate of 0.2 was observed for *K. oxytoca*. During the study in each unit one outbreak of nosocomial infections caused by K.pneumonia and MRSA, respectively, was seen. Increased rate of mucosal colonisation preceded both outbreaks. A molecular typing of mucosal and BSI isolates of *K. pneumoniae* and MRSA revealed a similar PFGE pattern.

Conclusion In NICU mucosal colonisation by MRSA and to lesser extent by *A. baumannii* and E.cloaceae carries a significant risk for BSI. Isolation of newborns colonised by microbes with the highest risk of subsequent BSI, should be implemented to avoid cross-colonisation by healthcare workers and thus prevent BSI.

P1091 Bloodstream infections among newborns in intensive care unit in a country network in Bogota, Colombia, 2001–2007

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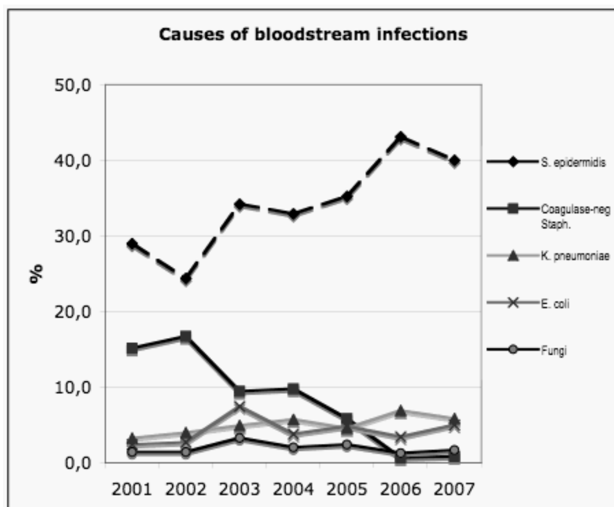
Background: Bloodstream infections are common among patients in the neonatal intensive care unit (NICU). Here we show the frequency of the microroganisms found in the blood of patients in the NICU belonging to a national network in Colombia.

Methods: A country network with 27 public and private tertiary hospitals from 3 cities was stablished. Laboratory information was transferred monthly to a central data base and compiled by the use of Whonet software (WHO, ver 5.4). Bloodstream infections were selected from those patients in the NICU.

Results: Between 2001 and 2007 (june), a yearly average of 689 bloodstream infections were detected among patients in the NICU. 60% to 70% of the isolates were Gram-positive, while near 30% were Gram-negative bacilli. In the figure, the annual percentage of the microorganisms more commonly isolated is shown. Of interest, 84% of the coagulase negative Staphylococci (CNS) were resistant to oxacillin and between 6.9% and 10.8% of *E. coli* isolates had a phenotype that suggested the presence of extended spectrum β-lactamases (ESBL). Such

phenotype was more frequently found in *K. pneumoniae* (41.1% to 51.1%). *Candida albicans* was the most frequently found yeast.

Discusion: Although oxacillin-resistant CNS predominated among bloodstream isolates, a high prevalence of Gram-negative bacilli was found, with a frequent expresion of ESBL. Fungal infections are not uncommon. Higher effords in infection control and antibiotic use should be made in order to diminished the number of resistant infections in high risk neonates.



P1092 Maternal and neonatal bloodstream infections: data from the Finnish Hospital Infection Program, 1999–2006

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Objectives: Maternal microbial flora may be a source for both maternal and newborn peripartum infection. The aim of this study was to evaluate the epidemiologic features and distribution of the causative pathogens of healthcare-associated bloodstream infections (BSIs) in women who were admitted for obstetric care and in neonates in Finnish hospitals during 1999–2006.

Methods: A prospective laboratory-based surveillance was performed in 9 Finnish acute care hospitals that participated in the Finnish Hospital Infection Program, including three tertiary care centres. Women in labour and infants with ages not more than 28 days were included. Infection-control nurses in each hospital regularly reviewed the laboratory database for positive blood culture results. Clinical information and microbiological data were recorded on a standardised case-record form. CDC definitions for nosocomial BSIs were utilised, both primary and secondary BSIs were included. We focused on ‘early-onset’ neonatal BSIs, referring to an infection with a positive blood-culture during the first 6 days of life.

Results: The study included 175 445 deliveries, of which 30 178 (17%) caesarean sections, and 177 987 live births. We identified 118 maternal peripartum BSIs (rate, 0.7 BSIs/1000 deliveries; range by year, 0.3–1.5/1000 deliveries), of which 67 (57%) occurred after a caesarean section, and 766 neonatal BSIs (rate, 4.4 BSIs/1000 live births), 288 (38%) of which were early-onset BSIs (rate, 1.6 BSIs/1000 live births; range by year, 1.1–2.4/1000 live births). The main causative pathogen of both maternal and neonatal early-onset BSIs was *Streptococcus agalactiae* (GBS) (Table). Of the infants with early-onset BSIs, 116 (40%) received intensive care and 16 (6%) died within a week after the positive blood culture; 7 (44%) of those had GBS disease. One of the mothers received intensive care, none of them died.

Conclusions: Maternal peripartum BSIs were rare and no fatal cases were detected. Neonatal early-onset infections were more common and led often to need of intensive care and even to death. Peripartum infections due to GBS, which was the most common pathogen, may be reduced by national prevention guidelines. Such guidelines were released in 2006 in Finland; future surveillance will show the impact of these guidelines.

Table: Distribution of causative pathogens in maternal and neonatal healthcare-associated bloodstream infections (BSIs) in Finland during 1999–2006

Maternal BSIs (N = 118)		Early-onset neonatal BSIs (N = 288)	
Streptococcus agalactiae	21%	*Streptococcus agalactiae*	33%
Staphylococcus aureus	14%	Coagulase-negative staphylococci	31%
Coagulase-negative staphylococci	11%	*Staphylococcus aureus*	11%
Streptococcus pyogenes	10%	*Escherichia coli*	6%
Viridans streptococci	9%	Enterococci	3%
Eschericia coli	7%		
Enterococci	7%		
Others	22%	Others	16%
Polymicrobial	3%	Polymicrobial	5%

P1093 *Pseudomonas aeruginosa* bloodstream infections: a 6-year retrospective review in a university hospital

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Objectives: *P. aeruginosa* bacteraemia (PAB) is a serious infection associated with a high mortality. Severity of underlying diseases, origins of infection, antimicrobial resistance and inadequacy of antimicrobial therapy have all been associated with an excess mortality rate. The objective of this study was to evaluate the epidemiology and the risk factors of PAB on the mortality rates among patients admitted to a 380-bed university hospital.

Methods: Retrospective cohort study of all patients admitted between 01/00 and 12/06 with a clinically significant PAB (at least one positive blood culture, excluding polymicrobic episodes of PAB). All medical charts were reviewed by means of a computer-based system. Variables studied were: demographic data, comorbidities (solid or haematological cancers, chronic lung, cardiac or liver diseases, solid organ transplant), immunosuppressive therapy, hospital events (nosocomial acquisition, admission to ICU, major surgical procedures), appropriateness of empirical antimicrobial therapy, mortality at day 7 and 30.

Results: 81 cases, 51 males (63%). Mean age: 64 yrs. Mean incidence of PAB: 3.9 per 100 bacteraemia episodes; 69% of PAB nosocomially-acquired. Underlying illnesses: 32% haematological cancers; 68% non haematological group (solid cancers 16%, COPD 11%, pulmonary grafts 7%, immunosuppressive therapy 16%), invasive diagnostic procedures 60%. Sources of PAB: respiratory 35%, primary 26%, urinary 15%. Number patients with appropriate empiric therapy 64% (100% with appropriate adjusted therapy). Crude mortality rate associated with PAB 31%; Attributable mortality at day 30 was 21% (18% at day 7); no significant difference in attributable mortality between patients with haematological illnesses (19%) and those with other comorbidities (22%); No differences in mortality rate according to the adequacy or inappropriateness of empirical antimicrobial therapy (20% vs 24%) but 2 out of 3 haematological patients with inadequate initial treatment died from their PAB.

Conclusions: the incidence of PAB has remained stable over 6 years. Two third of the episodes were of nosocomial origin. Haematological diseases, solid cancers and invasive procedures were the most common underlying diseases. Attributable mortality rate at day 30 was 21% and was not influenced by the type of underlying illnesses nor by the appropriateness of initial empiric therapy except in neutropenic patients with haematological cancers.

P1094 Analysis of risk factors for central venous catheter insertion site and catheter tip colonisation and for CVC-associated bloodstream infection in a randomised clinical trial

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Objective: To identify risk factors for skin colonisation prior to central venous catheter (CVC) placement and within 10 days after CVC insertion, for CVC-tip colonisation and for CVC-associated bloodstream infection (BSI).

Methods: As part of a randomised clinical trial, patients in the haematology units of the university hospitals Freiburg, Germany, and Basel, Switzerland and one cardiothoracic surgery unit (Basel) were enrolled after informed consent. Quantitative skin cultures were obtained from the insertion site prior to CVC placement and at regular intervals after CVC insertion. Cultures from the CVC tip on removal were undertaken (roll plate technique). In addition, data were collected on infection signs, therapy, nosocomial infections (according to CDC-criteria). The statistical analysis was performed using linear and logistic regression models.

Results: Four hundred patients (219 haematology, 181 surgery) with inserted CVC were enrolled in the trial (2002–05). Age >65 y and male gender were independent risk factors for skin colonisation prior to CVC placement in all patients and within the subgroup of surgical patients. Independent risk factors for skin colonisation within 10 days after CVC placement in all patients were: (i) colonisation prior to CVC placement (baseline colonisation), (ii) surgical patient, and (iii) male gender. Baseline colonisation of the skin at the insertion site was a strong predictor of CVC tip colonisation ($\geqslant$15 CFU). The underlying disease – haematological malignancy – was the strongest risk factor for CVC-related BSI.

Conclusions: The level of skin colonisation prior to catheter insertion predicts catheter colonisation and possibly subsequent infection. Elderly and male patients are at particular risk for colonisation of the skin and may be a target population for special precautions with meticulous care of the insertion site before and during catheterisation.

P1095 Needleless positive-pressure mechanical valve connectors: are they safe?

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Objectives: To determine whether the introduction of a needleless positive-pressure mechanical valve connector in adult and paediatric hematology-oncology units influenced the rate of catheter-related bloodstream infections (BSI) and the type of causative micro-organisms.

Methods: A mechanical valve (MV) connector system (CLC2000®, ICU Medical) was introduced in November 2005 and replaced miscellanous conventional open systems (COS) with a standard Luer-Lock removable cap. The main objective of this change was to reduce the use of heparin flushes in long-term tunnelled catheters (Hickman®, CR Bard).

The retrospective analysis included all patients with new Hickman catheters, inserted during two different study periods. The catheter-related BSI rate was observed during 6 months for each system and reported as number of BSI per 1000 catheterdays. The results with COS (January through June 2005) were compared with a similar period with MV-use (September 2006 through February 2007). Microbiological characteristics of bacteraemias occurring during each period were also compared. The hospital policy for care of Hickman catheters did not change over both study periods.

Results: During the COS-period, 39 Hickman catheters were inserted in 15 children and 24 adults with a total dwell time of 1544 and 1899 catheter days respectively. During the MV-period 60 Hickman catheters were placed in 13 children and 47 adults with a total dwell time of 1181 and 3223 catheterdays respectively.

Both in the paediatric and adult population, the rate of catheter-related BSI during the MV period was significantly higher than during the COS-period: resp. 11 vs 1.29 infections per 1000 catheterdays (p =.009) and 12.4 vs 6.32 infections per 1000 catheterdays (p = 0.019).

During the MV-period, the percentage of polymicrobial BSIs increased from 0 to 84.6% in children and from 8.3% to 21% in adult patients.

Conclusion: A significant increase in the rate of BSI was found among hematology and oncology patients with newly inserted Hickman catheters concomitantly with a change from a conventional open system to a mechanical valve connector, despite additional educational sessions regarding proper use of the device. The risk and type of BSI are clearly associated with the catheter cap design which promotes microbial contamination. The observed effects are more pronounced in children.

P1096 Late port-a-cath infection with *Enterobacter amnigenus* – An example of Gram-negative port-a-cath infection

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Objectives: To demonstrate the microbiology of infections of totally implantable venous access devices with emphasis on the importance of Gram-negative bacteria.

Methods: Case report and search of infectious disease charts

Results: We report a case of sepsis with *Enterobacter amnigenus* as a consequence of a totally implantable venous access device infection caused by contaminated saline solution. The indication for implantation of the port-a-cath system had been antibiotic treatment of diabetes-associated osteomyelitis of the foot which had been completed two years before onset of the actual disease. Explantation of the port-a-cath system together with adequate antibiotic treatment rapidly resolved the infection. *Enterobacter amnigenus* is a facultative Gram-negative pathogen associated with soil and water and displays a high percentage of natural antibiotic resistance. *Enterobacter amnigenus* infections have been associated with tap water, cardioplegia ice, thermometers, milk formulas and saline, albumine or heparine infusions. Port-a-cath infections with *E. amnigenus* have not been reported so far.

In a search of the charts of infectious disease consultations during the previous 5 years, 10 port-a-cath infections were identified, of which Gram-negative bacteria accounted for 60%. Gram-negative rods involved were *Enterobacter cloacae* (4), *Klebsiella pneumoniae* (2), *Pseudomonas aeruginosa* (1), *Enterobacter amnigenus* (1) and *Alcaligenes* spp. (1). 3 infections were polymicrobial. Only in 4 cases (40%), typical Gram-positive skin bacteria were identified in pure culture (*Staphylococcus aureus* (3), coagulase-negative streptococci (1)). Treatment of Gram-negative infections always required explantation of the intravascular device.

Conclusion: In conclusion it is crucial to re-evaluate the need for implantable venous access devices once the system is not used for therapy anymore. Furthermore, this case underlines that beside commonly isolated skin bacteria such as streptococcus and staphylococcus species, implantable intravascular catheter infections may also be caused by biofilm-producing Gram-negative bacteria, which should be considered in the empiric choice of antibiotics.

P1097 Under-reporting of occupational blood and infectious fluid exposure in a teaching hospital

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Background and Objectives: Occupational blood and infectious fluid exposure (OBE) poses a risk to healthcare workers (HCW). Implementation of safety precautions is crucial in preventing the transmission of hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV) in healthcare settings. Other key factors are HBV immunisation and appropriate post-exposure management.

The objective of the present study was to determine the proportion of HCW experiencing OBE, post-exposure managing and the proportion of vaccinated HCW in the largest tertiary and teaching hospital in Slovenia. The knowledge of HCW's hepatitis B surface antibody (anti-HBs) status was also assessed.

Methods: 251 HCW from surgical and non-surgical wards and laboratories completed a confidential questionnaire. All categories of HCW were assessed (doctors, nurses, laboratory workers and support personnel).
Results: 124 HCW (49.4%) experienced at least one OBE and only 63 of them (50.8%) reported the incident. HBV, HCV and HIV serology was performed in 44 cases for HCW (69.8%) and in 47 cases for the index patient (74.6%). Our personal observations as well as several studies showed that the most common reasons for non-reporting the OBE are the belief that the patient involved was not infected with HIV or HCV and that the wound was adequately disinfected. Significant amount of time and paperwork involved in reporting the incident are also a factor. Another important reason is the fear of being judged as well as the anxiety they would suffer while waiting for the test results. The data was further analysed according to category of HCW as shown in table 1.

Table 1. The analysis of the results of confidential questionnaire among HCW according to job category.

	all HCW No. (%)	physicians No. (%)	registered nurses No. (%)	nurses No. (%)	support personnel No. (%)	laboratory workers No. (%)
No.	251	51	38	88	8	66
HBV vaccination	229 (91.2)	49 (96.1)	37 (97.4)	82 (92.3)	3 (37.5)	58 (89.2)
anti-HBs titer following vaccination	106 (46.3)	22 (44.9)	23 (62.2)	22 (26.8)	3 (100.0)	36 (60.0)
Latest anti-HBs titer	227	49	36	80	6	56
0 IU/mL (non-responder)	15 (6.6)	2 (4.1)	1 (2.8)	5 (6.3)	0 (0.0)	7 (12.5)
>10 IU/mL	73 (31.9)	23 (46.9)	16 (44.4)	7 (8.8)	0 (0.0)	27 (48.2)
unknown	83 (36.2)	18 (36.7)	14 (38.9)	42 (52.5)	1 (16.7)	8 (14.3)
never determined	56 (24.5)	6 (12.2)	5 (13.9)	26 (32.5)	5 (83.3)	14 (25.0)
OBE	124 (49.4)	34 (66.7)	28 (73.7)	44 (50.0)	2 (25.0)	16 (24.2)
Reporting of OBE	63 (50.8)	15 (44.1)	17 (60.7)	22 (50.0)	1 (50.0)	8 (50.0)
HBV, HCV and HIV screening of HCW	44 (69.8)	11 (73.3)	12 (70.6)	13 (59.1)	1 (100.0)	7 (87.5)
HBV, HCV and HIV screening of index patient	47 (74.6)	13 (86.7)	15 (88.2)	12 (54.5)	1 (100.0)	6 (75.0)
Anti-HBs titer following reported OBE:	54 (85.7)	12 (80.0)	15 (88.2)	19 (86.4)	1 (100.0)	7 (87.5)
0 IU/mL (non-responder)	5 (7.9)	1 (7.1)	2 (12.5)	0 (0.0)	0 (0.0)	2 (25.0)
>10 IU/mL	28 (44.4)	9 (64.3)	8 (50.0)	8 (38.1)	0 (0.0)	3 (37.5)
unknown	27 (42.9)	4 (28.6)	6 (37.5)	13 (61.9)	1 (100.0)	3 (37.5)

HCW – healthcare workers. OBE – occupationa1 blood and infectious fluid exposure; HBV hepatitis B virus. HCV – hepatitis C virus. HIV – human immunodeficiency virus; anti-HBS serum level of hepatitis B surface antibody.

While the majority of HCW received HBV immunisation (91.2%), the serum concentration of anti-HBs following immunisation was assessed in 106 (46.3%) HCW only. 15 (6.6%) HCW were non-responders and 73 (31.9%) reported adequate serum levels. Surprisingly, over one third of HCW (36.2%) did not know their serum levels of anti-HBs, while for 56 (24.5%) their response to immunisation was never confirmed.
Conclusion: The rate of OBE in this pilot study and inadequate post-exposure measures are disturbing. Our data has shown that better educational programs are needed to increase awareness about the risk and prevention of OBE among the HCW. HCW should be encouraged to undergo testing to determine adequate response to HBV vaccination and most importantly to improve the compliance with the recommended measures following OBE.

P1098 Healthcare workers' exposure to blood-borne pathogens in Lebanon

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Objectives: Study the pattern of occupational exposure to blood and body fluids (BBFs) at a tertiary care hospital in Lebanon for 17 years. This is the first study to provide baseline data aiming to introduce the suitable interventions.
Methods: This study retrospectively reports a 17 year experience (1985–2001) of on-going surveillance of HCW exposure to BBFs at a 420 bed academic tertiary care hospital. Exposures were defined as any eye, mouth, or other mucous membrane, non-intact skin, or parenteral contact with blood, or other potentially infectious body fluid. The endpoints were the annual number, nature, and cause of accidents, as well as the HCW's status, injury tool, site, and source.
Results: 1590 BBF exposure-related accidents were reported to the Infection Control Office. The trend showed a decrease in these exposures over the years with an average ±SE of 96±8.6 incidents per year (figure). In the last 6 years, the average rate of BBF exposures was 0.57 per 100 admissions per year (average of needle stick injuries alone was 0.46 per 100 admissions). Needle sticks accounted for 75% of the injuries, sharp objects for 10%, splash and spill for 3%, and in 12% it was not clearly specified. The highest occurrence of these exposures was on the ward (35%) followed by the operating room (17%) and intensive care units (15%). The reason for the incident, when stated, was attributed to a procedural intervention (29%), improper disposal of sharps (18%), to recapping (11%), and to other causes (5%). The patient or source of incident was known in 64% of cases. For studying rates of exposure among the different occupational categories, the year 2001 was taken as an example. 13% of house officers, 9% of medical students, 8% of attending physicians, 5% of nurses, 4% of housekeeping, 4% of technicians, and 2% of auxiliary services employees had a BBF exposure during that year.

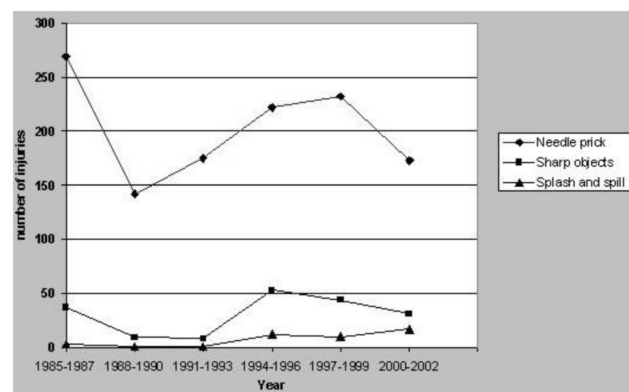


Figure 1. Incidence of blood and body fluids exposure over the years by type.

Conclusion: The current study showed that exposure of HCWs to BBPs remains a problem. This can be projected to other hospitals in the country and raises the need to implement infection control standards as part of a complete system where the culture of safety becomes an integral part of the organisation. Prospective studies are needed on a yearly basis to identify high risk groups and develop focused interventions.

P1099 Underreporting of accidental blood exposure in Belgian hospitals

E. Leens, C. Suetens on behalf of the Belgium National Steering Group of Accidental Blood Exposure

Objectives: In order to estimate the percentage of accidental blood exposures (ABE) not reported to the occupational health department in Belgian hospitals, a national underreporting study was carried out.
Methods: In May 2006, the Scientific Institute of Public Health invited 55 hospitals participating to the national surveillance of ABE (EPINet) to the study. Twenty percent of the healthcare workers (HCW) filled out a questionnaire anonymously during the annual consultation of occupational health (OH). They were asked how many percutaneous injuries (PI) or blood or body fluid exposures (BFE) occurred during the last month and the last year, and if so for how many ABE a registration formular (EPINet or OH insurance) was filled out.
Results: Till 28 February 2007, a total of 3311 questionnaires from 25 hospitals was received. For the recall period of 1 month 103 PI occurred in 78 HCW, of which 52 PI were registered by 38 HCW (underreporting of 49.5%, Table 1). BFE were more frequent in 1 month, but were much less registered: 165 HCW mentioned 452 BFE in the study, but only 3 cases (by 3 HCW) were declared to the surveillance or OH insurance system (99.3%). For the recall period of 1 year the percentage of non-reported injuries was 45.2% of the PI and 96.4% of the BFE.

Table 1: Percentage not reported ABE in Belgian hospitals, 2006

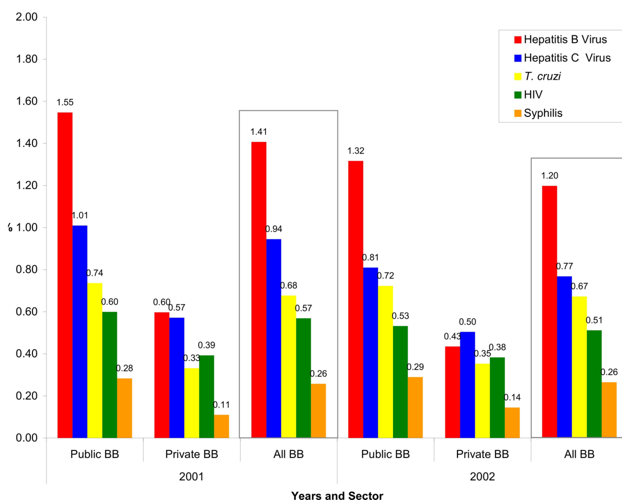
	% not reported (95% CI)	
	last month	last year
PI	49.5% (39.5–59.5%)	45.2% (40.8–49.6%)
BFE	99.3% (98.1–99.9%)	96.4% (95.1–97.4%)

Conclusion: Results show that half of the PI was not declared by the HCW. The underreporting rate in Belgian hospitals is similar to average rates found in literature. The high underreporting rate for BFE was possibly related to a misinterpretation of the definition. Based on the results of this study and the PI rates of the national surveillance, the annual number of PI in Belgian hospitals could be estimated at approximately 10800 PI.

P1100 Seroprevalence of blood-borne infections among blood donors in Venezuela, 2001–2002

J. Benitez, A. Rodriguez-Morales, M. Arria (Maracay, Trujillo, Caracas, VE)

Objectives: From the data collected at the Ministry of Health from whole nation public and private blood banks (BB) find out the proportion of blood units discarded for being seropositive HBV, HCV, HIV, syphilis and *Trypanosoma cruzi*, and the seroprevalence of these infections among their donors.
Methods: ELISA serological testing was done with 715,393 donors seen at 535 blood banks between 2001 and 2002. Samples that were repeat reactive (RR) with the ELISA underwent supplementary Western blot (WB) testing.
Results: Of the 715,393 blood banks donors, 9,294 of them (1.3%) were positive for syphilis (VDRL), 6,107 (0.85%) for HBV (anti-HBc), 4,825 (0.67%) for *T. cruzi*, 3,859 (0.54%) for HCV and 1,870 (0.26%) for HIV. In the Figure a summary of the seroprevalence of these infections, among public and private blood banks, per year, is presented.



Conclusions: Although syphilis or *Treponema pallidum* infection was the most important blood-borne disease found in this study, the seroprevalence found is lower than others reported in other countries in the region (e.g. Goiânia, Brazil, 1989, 4.1%). For HBV and HCV, the seroprevalence estimates were also similar than those found in other countries in the region (such as Río de Janeiro, Brazil, where in 2005 the anti-HBc was 2.05% and 0.79% in 2004 for HCV), indicating high rates of infection by HBV and HCV and a persistent risk of HBV and HCV transmission by transfusion. For Chagas disease is also lower than others reported in other countries in the region (e.g. Goiânia, Brazil, 1989, 3.3%). Finally for HIV the found seroprevalence is similar to that reported in Mexico (Irapuato, Mexico, 2003, 0.24%). But as seen herein, those seroprevalences are regional, and our report is nationwide. Further epidemiological research is expected. [Figure. Seroprevalence of Blood-borne Infections among Blood Donors in Public and Private settings in Venezuela, 2001–2002]

Infection control and nosocomial infections

P1101 Skin care and use of colouring- and perfume-free products for hand hygiene: important foci for hand hygiene promotion

A. Conrad, I. Poser, S. Wenzler-Röttele, A. Pfister-Wartha, M. Dettenkofer (Freiburg, DE)

Objective: Hand disinfection with an alcoholic hand rub is the most effective measure for preventing nosocomial infections. However, frequent use of hand hygiene products may cause skin irritation, which, in turn, results in poor hand hygiene compliance. Additionally, many products contain colouring and perfumes which hold a certain risk of sensitisation, although they do not contribute to efficacy. The objective was to improve hand hygiene by promotion of skin care and introduction of colouring and perfume-free products for hand hygiene.
Methods: A campaign to improve hand hygiene at the University Medical Center Freiburg, Germany, was initiated along with the introduction on 11 intensive care units and hematology wards of a colouring and perfume-free product line (disinfectant and soap). The intervention was accompanied by 15 min training sessions on hand hygiene indications, technique and skin care. Before and after a 6-week intervention phase, opinion on hand hygiene and acceptance of products were evaluated using structured questionnaires.
Results: Of a total of over 1200 questionnaires distributed 54% (of 660) were completed before and 41% (of 611) after the intervention (by nurses and by physicians). Hand hygiene was judged to be of high relevance to patient safety by 76% of the respondents before and by 85% after the intervention. Before the intervention, 36% had no preference for any hand disinfectant, and used various products according to availability. However, after the intervention 41% preferred the colouring and perfume-free alternatives. This figure was dependent on gender since almost 50% of women preferred the test products compared to less than 30% of the men. The colouring and perfume-free products were generally tolerated as well as conventional preparations. Only 3% of the respondents complained about malodour of the test preparations. The most frequent complaint associated with hand hygiene preparations in general was dryness of the skin (over 70%). Healthcare workers' use of skin care rose from 52% to 64%.
Conclusions: Skin care and use of colouring and perfume-free products are important foci of hand hygiene promotion. Taking into account that staff tends to prefer products they know, this project successfully introduced a colouring and perfume free-product line which was well tolerated. Since colouring and perfume are dispensable, in future, only colouring and perfume-free hand hygiene products should be used in daily routine.

P1102 Effectiveness of hand-cleansing agents for removing meticillin-resistant *Staphylococcus aureus* from contaminated skin

Z. Muszynski (Poznan, PL)

Objectives: The effectiveness of hand-cleansing agents in removing a hospital strain of meticillin-resistant *Staphylococcus aureus* from artificially contaminated hands of ten volunteers was studied.
Methods: The products used were plain liquid soap, ethyl alcohol 70% (by weight), 10% povidone-iodine liquid soap (PVP-I), and 4% chlorhexidine gluconate detergent in water. The experiments were performed using a Latin square statistical design, with two 5 x 4 randomised blocks. The removal rates of *S. aureus* cells from contaminated fingertips were estimated by analysis of variance, the response variable being the logl0, reduction factor (RF), ie, logl0 of the initial counts minus logl0 of the final counts. In the first and second

blocks, the fingertips of the volunteers were contaminated in mean with 3.95 logl0 colony-forming units (CFU) light-contamination hand and 7.24 logl0 CFU heavy-contamination hand, respectively.

Results: In the first block, there were significant diferences between treatments (P < 0.05). The 10% PVP-I (RF, 3.96) and 70% ethyl alcohol (RF, 3.95) had significantly higher removal rates than plain liquid soap (RF, 2.44) and 4% chlorhexidine (RF, 2.98). In the second block, 10% PVP-I (RF, 4.55) and 70% ethyl alcohol (RF, 3.80) also were significantly more effective than plain liquid soap (RF, 1.95) and 4% chlorhexidine (RF, 2.24; P < 0.05). Plain liquid soap was significantly more effective than 4% chlorhexidine detergent.

Conclusions: The results suggest that 10% PVP-I and 70% ethyl alcohol may be the most effective hand-cleansing agents for removing meticillin-resistant *S. aureus* strain from either lightly or heavily contaminated hands.

P1103 Hand hygiene after toilet visits

D. van der Vegt, A. Voss (Nijmegen, NL)

Introduction: Compliance with hand hygiene in the healthcare setting, is generally low (on average 40%), despite the fact that good hand hygiene still is one of the most important measures to prevent nosocomial infections. Since the main indications for hand hygiene differ between healthcare and non-healthcare settings no comparison has been done.

Objective: Aim of the present study was to investigate the degree of compliance with hand hygiene after toilet visits in and outside the healthcare setting.

Methods: We observed different group of healthcare providers (hospital/laboratory personnel and participants of ECCMID 2007), and users of public lavatories along motorways with regard to their compliance with hand washing after toilet visits

Results: Compliance with hand hygiene after toilet visits was, 46%, 84% and 75%, respectively. Obviously, healthcare workers show a different compliance with hand hygiene in and outside the healthcare setting.

Conclusion: While the comparison of these different groups most certainly is "flawed" and "biased" it is interesting to see, that hand hygiene compliance after toilet visits is the lowest in the healthcare setting, while another group of healthcare-workers (ECCMID participants) had the highest compliance. Possibly, the feeling of a "miasmic" environment (presumed "dirty" public toilets) triggers hand hygiene compliance, whereas the "clean" hospital environment may jam the trigger to do so.

P1104 Engaging more than 300 healthcare facilities from Europe to participate in the testing of the WHO multimodal hand hygiene improvement strategy

G. Dziekan, J. Storr, B. Allegranzi, A. Leotsakos, C. Engineer, H. Sax, D. Pittet (Geneva, CH)

Healthcare-associated infections (HAI) affect hundreds of millions of people worldwide and are a major global patient safety issue. During healthcare delivery, in most cases pathogens are transmitted by healthcare workers' hands.

Prevention of HAI is the target of the First Global Patient Safety Challenge, "Clean Care is Safer Care", launched in October 2005 by the WHO World Alliance for Patient Safety. WHO "Guidelines on Hand Hygiene in Health Care (Advanced Draft)" have been produced together with a multimodal implementation strategy to improve hand hygiene at the bedside. The strategy is currently being tested at selected hospitals within each of the six WHO regions worldwide to validate the guideline-recommendations.

Objective: To acquire feedback on the feasibility of implementing a multimodal HH improvement strategy in non-selected healthcare facilities across the socioeconomic and cultural range, including highly developed and least developed countries.

Methods: The WHO programme has set up a web-based community platform to allow every HCF in the world to register and participate as a Complementary Test Site. Registered facilities have access to a suite of 40 implementation, training and evaluation tools, including a detailed "Guide to Implementation".

Results: Within the first ten months more than 300 healthcare facilities (hospitals, ambulatory clinics, long-term care facilities) from European countries have registered either as individual healthcare settings or represented by national/sub-national coordinators. Several networks of hospitals have formed as part of national or sub-national campaigns following a pledge by the Ministry of Health to address the growing burden of HAI. In Italy 143 hospitals are engaging in the HH improvement strategy, in Switzerland 116 hospitals, in Spain and Cataluña 22 hospitals are participating. National coordinators have adapted and translated the provided material to implement the multimodal HH improvement strategy to ensure engagement and sustainable effect.

Conclusion: Political awareness and leadership can mass-mobilise healthcare facilities to engage in quality improvement strategies offered at a global level. Coordination at national or sub-national levels is needed to provide a consolidated approach to ensure sustainability.

P1105 Foul places to trigger clean behaviour

B. Wiesman, M. van Schie, A. Voss (Delft, Nijmegen, NL)

Introduction: Compliance with hand hygiene is a continuous problem within healthcare settings. Multimodal interventions are recommended to improve healthcare workers compliance, including those focussing the attention on hand hygiene by visual reminders, such as "speaking walls" (posters).

Objective and Method: We were looking for new and innovative ways of placing reminders for hand hygiene within the hospital setting. Aim of the study was to evaluate the effect of "promotional toilet paper" on hand-rub use in hospital wards. The hand-rub use was measured using dispensers equipped with sensors that allowed wireless transfer of data to a central computer. A software was developed to translate the different dispenser motions into hand-rub use data (NewCompliance®)

Results: Placement of promotional toilet paper significantly increased the hand rub use of healthcare workers. The effect decreased after some time and could be repeated after introduction of a new design.
Conclusion: This study shows that a simple, low cost intervention, namely the placement of toilet paper containing motivational messages, may have a significant impact on the use of hand disinfectants and may therefore become a part of the multimodal interventions to increase hand hygiene compliance. Furthermore, automated data collection of hand rub use can be a useful instrument to evaluate the effect of interventions to increase hand hygiene compliance. As with many interventions, the effect loses its impact over time, but can be boosted by a change of motifs. Obviously, in the case of motivational toilet paper, clean behaviour can be triggered in foul places.

P1106 Wristwatches as potential sources of hospital-acquired infection

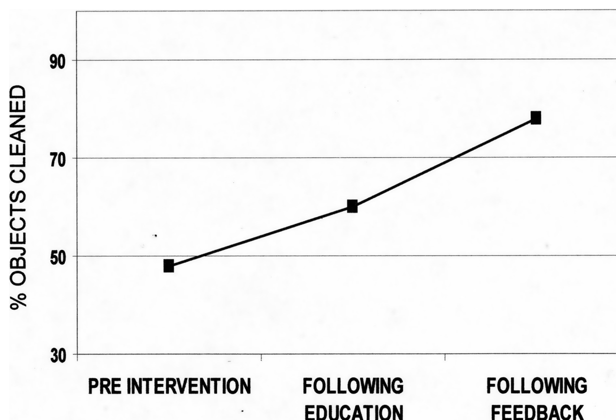
A.R. Jeans, J. Moore, C. Nicol, C. Bates, R.C. Read (Sheffield, UK)

Objectives: To measure the rate of bacterial hand and wrist contamination, particularly by *Staphylococcus aureus*, amongst healthcare workers (HCWs) who wear wristwatches.
Methods: Two consecutive cohort studies of wristwatch wearers and non-wristwatch wearers were conducted amongst samples of HCWs. In the first study (n = 300), wrists were sampled by skin swabs and hands by direct plate inoculation. In the second study (n = 355) this was done after the HCW was asked to remove their watch immediately prior to sampling.
Results: *S. aureus* was found on the hands of 25% of the wristwatch wearers and 22.9% of the non-wristwatch wearers in the first study. Watch wearers had higher counts of bacteria on their wrist ($p < 0.001$), but not on the hands.
In the second study, removal of the watch prior to sampling resulted in increased counts of bacteria on both hands as well as the watch wrist compared with non-watch wearers ($p < 0.001$).
Conclusion: Wristwatch wearers have an excess of bacterial contamination of the wrists. They do not have an excess of hand contamination unless the watch is manipulated.

P1107 Improving patient area disinfection cleaning in 34 acute care hospitals

P. Carling on behalf of the Healthcare Environmental Hygiene Study Group

Objectives: It has become increasingly recognised that microbial contamination of the patient's immediate environment plays a significant role in the transmission of many healthcare associated pathogens. Despite the recent recommendation that hospitals experiencing increased transmission of MDROs "Monitor (i.e., supervise and inspect) cleaning performance to ensure consistent cleaning and disinfection of surfaces in close proximity to the patient and likely to be touched by the patient and healthcare professionals" (CDC –2006; Category 1B), there currently exists no practical means of evaluating and improving the effectiveness of these activities.
Methods: An invisible fluorescent targeting method was used to confidentially evaluate the cleaning of 14 standardised high-touch objects. Rooms were marked following terminal cleaning and re-evaluated after one to two patients had occupied the room and it had been again terminally cleaned.
Results: 1511 rooms and 19,361 objects were evaluated in 34 hospitals ranging in size from 25 to 721 beds. The mean proportion of objects cleaned was 48.5% (95% CI 42.9 to 47.5) prior to educational interventions. While sinks and toilet seats were relatively well cleaned (79.5%), consistently low rates of cleaning were documented for objects at high risk for microbial contamination including bedpan cleaners, toilet area handholds, bathroom and room doorknobs and bathroom light switches (23.3%). Following educational programs and process improvement feedback the cleaning of 448 rooms and 5386 objects were evaluated in the same manner. Overall thoroughness of cleaning improved to 77% (95% CI 78.5% to 89.2%) or 62% from baseline. All hospitals realised significant improvement over pre-intervention results ($P \leqslant 0.0001$).
Conclusion: The use of an objective targeting method in 34 acute care hospitals disclosed substantial opportunities for improving room cleaning/disinfecting activities. Following educational interventions and ongoing performance feedback to the environmental services staff, highly significant enhancement of near-patient environmental cleaning was objectively documented in all hospitals.



P1108 Bactericidal effect of disinfectants on *Chryseobacterium meningosepticum*

M. Ceyhan, E. Kilic, I. Yildirim, M. Ozalp, A.B. Cengiz, A. Bagdat, C. Barin (Ankara, TR)

Three outbreaks caused by *Chryseobacterium meningosepticum* on 5 different wards of a tertiary care paediatric hospital (Hacettepe University, Ankara) with 7 different positive environmental samples were observed between July 2006 through January 2007.Antibiogram typing and ERIC-PCR revealed that all isolates were epidemiologically related. *C. meningosepticum* was isolated from several dry environmental samples(electric button, computer keyboard, phone, door knob, AMBU bag) in addition to an infant formula. Disinfection and cleaning of the environmental surfaces were started in twice daily manner. However, we could not find any precise route of disinfection and type of chemical to use in the literature. This study was planned to evaluate the efficacy of hospital disinfectants against *C. meningosepticum*.
Polyhexanide (Hexanios G+R, France), didecyl dimethylammonium chloride (Surfanios, France), 4% chlorhexidine digluconate (Dermanios scrub chlorhexidine, France), 6.66 g benzalkonium chloride plus 3.33g alkyldimethylammonium chloride (Cleanisept spray, Germany) and solution containing 42 g ethanol, 0.05g didecyl dimethylammonium chloride (Descosept AF, Germany) were tested for bactericidial activity against *C. meningosepticum* isolated from the door-knob according to the principles of EN 1040 standarts. Surviving colonies were enumerated and expressed as colony forming units per milliliter. The reduction rate was calculated as the expression of the disinfectant efficacy according to the following formula $\log_{10}$ reduction = $\log_{10}$ control count – $\log_{10}$ disinfection count. Log_{10} reductions of >5 were taken as an indication of satisfactory bactericidal activity. Susceptibility testing was performed using the quantitative suspension test.
The test results are presented as $\log_{10}$ reduction of the bacteria after 5 and 60 minutes of contact. All studied disinfectants were uniformly bactericidal (effective in producing a >5-log reductions) in 5 and 60 minutes at their in-use concentrations. The efficacy of Hexanios G+R and Surfanios were tested three different concentrations including in-use concentrations, other disinfectants were studied for in-use concentrations only.
The efficacy of surface disinfection and cleaning in reducing microbial loads and their dissemination is one of the critical points to control

outbreakprevent prevention of nosocomial infections. All of the studied disinfectants can be used in cleaning of the environmental surfaces during nosocomial *C. meningosepticum* outbreaks.

P1109 A new experimental model designed to evaluate the antifungal effect of UVC radiation against medical airborne fungi

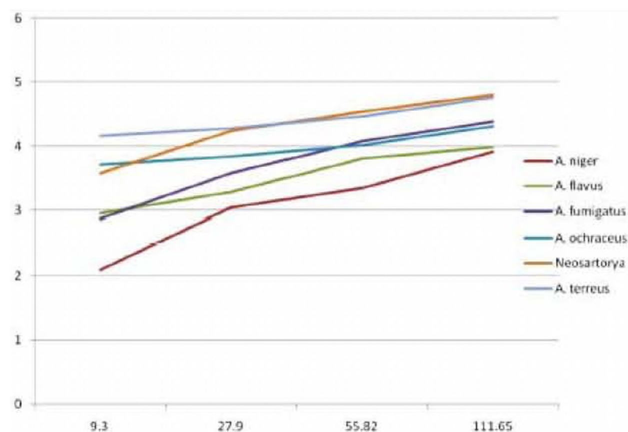
L. Malic, I. Ailincai, M. Mares (Iasi, Bucharest, RO)

Objectives: The aim of this paper is to propose a new and reproducible experimental model designed for evaluation of fungicidal effect of UVC radiations with a wavelength of 254 nm against medical airborne fungi, and its validation.

Methods: We achieved a testing module with a continuous monitoring and setting of work parameters (temperature, humidity, exposure time) and a complex device for generating UVC radiations and measurement of dose which offer the possibility to perform experiments in biological and radiological safety conditions. In order to validate this device, we performed some experiments with six strains of airborne fungi (*Aspergillus niger*, *A. flavus*, *A. fumigatus*, A. ochraceus, *A. terreus* and Neosartorya fischeri). For testing we used fungal spores suspensions in saline solution with 0.05% Tween 80, having a final concentration of 10^6 CFU/ml. From each suspension, 0.1 ml have been plated onto Potatoes Dextrose Agar and after drying, the Petri dishes were exposed to UV radiations in a dark field for various periods of time (final doses: 9.3, 27.9, 55.82 and 111.66 mWs/cm^2). After exposure, the plates were rapidly covered with black paper and transferred to incubation in order to prevent DNA photoreactivation. Each test was done in triplicate. After 72–96 hours of incubation, the intensity of fungicidal effect has been calculated using the Ir (decimal logarithmic reduction index).

Results: The Ir values have a linear increment for all tested strains while the UV doses increase. The arithmetic means of Ir were: 3.16 – *A. niger*, 3.58 – *A. flavus*, 3.89 – *A. fumigatus*, 3.96 – A. ochraceus, 4.29 – N. fischeri, 4.41 – *A. terreus*. The difference of susceptibility for these six species to UVC radiation is not statistical significant ($p \gg 0.05$), but the increase of p values when *A. terreus* is compared with the remaining strains emphasise some minor differences. Also, no significant differences were observed between the three series of experiments ($p > 0.05$) demonstrating the validation of our model as a reproducible one.

Conclusion: We proposed and verified a new experimental model for studying the fungicidal effect of UVC radiations. The tests performed using six strains of spore-forming fungi have proved that our model is a safe, easy to perform and reproducible one. This model may be used for dose evaluation in order to determining the appropriate CFU number reduction for medical airborne fungi in hospitals, pharmaceutical, food and feed industries.



P1110 Point-of-use filters to prevent fungal contamination of hospital water

A. Onken, P. Gaustad, T.G. Abrahamsen, A. Warris (Rud, Oslo, NO; Nijmegen, NL)

Objectives: To investigate whether the installation of point-of-use filters could retain the fungal contamination of the hospital water at the Rikshospitalet, Univ. of Oslo, Norway. Previously, we showed that the hospital water of the paediatric oncology department was contaminated by fungal organisms. Furthermore, hospital water has been suggested as a route for transmission of invasive filamentous fungal infection and preventive measures are needed to protect immunocompromised patients.

Methods: In April 2005 water samples were taken from 10 showers (hot water) and 10 sink taps (cold water) at the paediatric oncology ward, including four BMT units. The samples were collected in sterile glass bottles on three different days in an 8-day period. Then, point-of-use filters (Pall-Corporation®) were installed on the taps and showerheads. Sixty samples were taken from showers and sink taps before installation point-of-use filters, 20 samples were taken with the filters in place. The collected samples were filtered through sterile filters, pore size 0.45 μm. The filters were deposited on Sabouraud glucose agar, incubated for 7 days and examined for fungal species and identified by macroscopic and microscopic characteristics. *Aspergillus* isolates were identified to the species level.

Results: Filamentous fungi were recovered from 97% (58 of 60 samples) of the water samples before installation of the point-of-use filters. *Aspergillus fumigatus* was isolated in 21 of 60 samples (35%).

On average, 2.9 colony forming units (cfu)/ 500 ml of filamentous fungi were recovered, *Aspergillus* sp. 0.7 cfu/ 500 ml, *A. fumigatus* 0.6 cfu/ 500 ml.

After the installation of local filters no moulds were recovered (20 samples), but the filters were rapidly occluded within 1 or 2 days. Electron microscopy showed that the surface of the occluded filters was completely covered with large amounts of particulate colloidal material.

Conclusions: The installation of point-of-use filters retained the fungal contamination of the hospital water completely. Due to a high level of organic material in the water, the filters were blocked after being in use for only 1 or 2 days. Pre-filtration at the inlet of the water into the paediatric oncology department should be carried out to get rid of the organic materials. At present, it is not clear, if this is feasible. Our findings indicate that point-of-use filters might be useful unless the water contains much organic material blocking the filters.

P1111 Healthcare professional preferences in educational strategies against antimicrobial resistance in a developing country

J.S. Castillo, R. Sánchez, G. Buitrago, A.L. Leal, J.A. Cortés, C.A. Álvarez on behalf of GREBO

Background: Infection control strategies are based on behaviour understanding and education. Educational strategies tend to fail due to inability to use adequate methodologies or didactic approaches to healthcare workers. Here we try to find out the educational preferences for healthcare workers in Bogotá, Colombia.

Methods: We perform a cross sectional survey in a 50 hospitals network with 11626 hospital healthcare workers. Estimated sample size was collected with a multi-stage sampling strategy. Interviews were done using a format defined by an expert panel. Preferences were scored in a likert scale. An electronic capture application (Teleform™) was used, all descriptive analysis were carried out in Stata 9.0 (licensed version).

Results: 598 healthcare givers were included. All work shifts were represented. Sample had 33.1% physicians, 41.6% nurses and 25.3% other healthcare givers. 65.7% reported only graduate instruction. Inadequate self reported knowledge was 33.8%, no differences between medical and non medical professionals were not found. Educational activities were a need for therapeutic and preventive areas. How to give information to patients was a second motivation for physicians. Workshops were the most preferred educational activity (Figure 1). The

main argument to choice an strategy were in decreased order the capacity to improve knowledge, availability, no special technology requirements, finantial support, time and effort demanding activities (Figure 2).

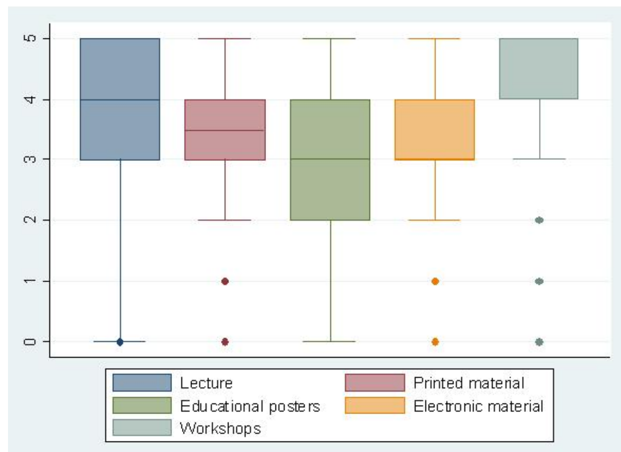


Figure 1. Preferences in educational methodology

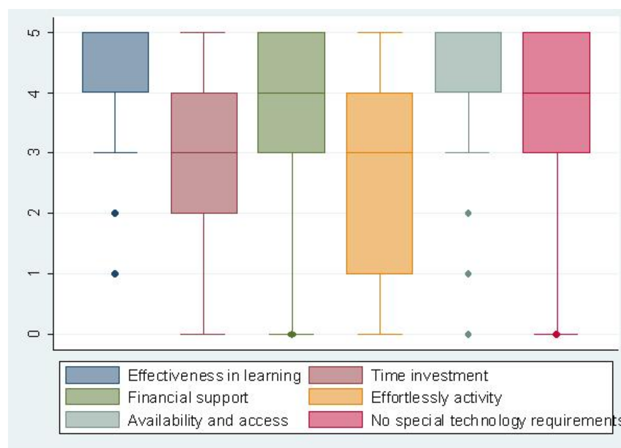


Figure 2. Key features for activity selection

Conclusions: No differences were found in preferences for medical and nonmedical target. Self reported preferences are of great relevance to design an educational intervention to contain antimicrobial resistance. Participatory interventions as workshops are the favorite ones.

P1112 Influence of a hygiene promotion programme in infection control on an intensive care unit

J.F. Murcia-Payá, E. García-Vázquez, A. Melgarejo-Moreno, J. Martínez-Hernández, F.J. Gil-Sánchez, M. Canteras, J. Gómez-Gómez, J. Allegue (Cartagena, Murcia, ES)

Objectives: The inconsistent application of hygiene measures by healthcare workers accounts largely for the epidemic dissemination of nosocomial infections (NI); our aim is to know the prevalence of NI on an Intensive Care Unit (ICU) and to assess the influence of a hygiene promotion programme in the rate of NI.

Patients and Methods: The efficacy of a hygiene promotion programme to prevent NI was assessed in our ICU during 1-year period; initially, a prospective and observational study of NI cases on the ICU during a 6 month period (PI) was done; epidemiological and clinical variables as well as information about risk factors related to NI were assessed. These data were compared to those corresponding to a second prospective and observational study period (PII) during which a health-workers hygiene promotion programme was established. Education of hospital staff was an essential component of the programme aimed to predispose healthcare workers to adopt hand hygiene behaviour (poster campaign and educational meetings and handouts), reinforce and enable the behaviour (provision of an alcohol hand rub beside each patient's bedside). Antibiotic policies and ICU staff were the same during the two periods.

Results: 395 patients were assess during PI and 411 in PII; there were not statistically significant differences on epidemiological or clinical variables during the two periods; there was no difference in risk factors related to NI (underlying conditions, severity at admission, rate of urgent surgeries, invasive techniques, ICU length of stay, mechanical ventilation, etc.). The rate of infection in PI was 26% and 16% in PII ($p < 0.05$). Hand-washing rate was significantly higher in PII than in PI (previous to patient care: 45% and 35% respectively and post patient care: 63% and 51%, respectively)

Conclusion: Patient's features during PI and PII were similar; there was a significant increase in ICU staff hand-hygiene compliance during the educational phase; the incidence rates of NI during the pre-programme period (PI) were significantly higher than during the programme period.

P1113 Peripheral venous cannulation in surgical patients: could we improve?

A. George, S. McKain, K. Shute, K. Swarnkar (Newport, UK)

Aims: More than 90% of all patients admitted to hospital will have a peripheral cannula inserted. Complications associated with this procedure include extravasation, thrombosis and infection. We aimed to investigate both the compliance of peripheral venous cannulae insertion with national guidelines as well as assess the indications, maintenance and complications associated with its use.

Methods: 40 randomly selected surgical patients with a peripheral venous cannula in situ were monitored daily for 8 days or until removal of the cannula. A non-interventional study was undertaken by gathering information on all aspects of care including site, size, use and indications, level of documentation, status of dressings, infection/ thrombosis risk using the VIPS (Visual Infusion Phlebitis Score) as well as cannula changes.

Results: Analysis demonstrated poor compliance with choice of non-dominant hand, cannula size, continuing indications for use. 96% of cannulae had no documentation regarding its insertion, removal or changes. 40% of the inserted cannulae were never used. Compliance of cannulae care and maintenance was poor with most of the cannula dressings becoming contaminated with blood, pus or fluid or the cannulae being inappropriately secured with non-sterile adhesive tape and bandage dressings – inappropriate and contaminated dressings showing a strong association with infection. A high rate of infected cannulae (17%) and a non-compliance with best practice for cannulae removal was seen even when the VIPS score was 2 or more (indicating active thrombophlebitis) with a significant group being left in and used even in the presence of active infection. Most cannulae were left in-situ for 48 or more hours after its use had stopped with a significant minority being left in for more than 120 hours.

Discussion: This study shows that though definite protocols exist for the use, maintenance and removal of peripheral cannulae, they tend to be eclipsed and sidelined. The insertion of these small peripheral intravenous devices poses the potential of becoming a major source of infection if their use and maintaince is not monitored. An overall improvement in the awareness of peripheral intravenous cannulae care, use and documentation is essential to ensure best practice in surgical patients.

P1114 Simple procedures to reduce *Staphylococcus epidermidis* adhesion to indwelling medical devices

C. Sousa, P. Teixeira, R. Oliveira (Braga, PT)

Objectives: The aim of this work was to develop expedite procedures to reduce *Staphylococcus epidermidis* adhesion to materials normally used

in indwelling medical devices such as catheters and prosthesis using only a pre-contact of the material surface with two conditioning substances: heparin and gentian violet. Heparin is a proteoglycan with strong anticoagulant activity, normally used to minimise thrombus formation, and gentian violet is a triphenylmethane dye that is normally used as a biological stain and as antiseptic agent.

Methods: Three clinical *S. epidermidis* strains were studied: 9142-M10, 9142 and IE186. Acrylic and silicone were used as substratum. Coupons (2 cm × 2 cm) were immersed in heparin or in 1% gentian violet solution, left to soak for 2 hours and left to dry overnight at 21 ºC. For the adhesion assays, the coupons were placed in 6 well tissue-culture plates with 4 mL of a cell suspension (1×10^9 cells/ml), for 2 hours, at 37ºC and 120 rpm. Each coupon was then stained with 4,6-diamino-2-phenylindole (DAPI) solution and adhered cells were visualised under an epifluorescence microscope and enumerated with appropriated software. The coupons with adhered cells were also observed by scanning electronic microscopy (SEM).

Results: The results of the adhesion assays point to a significant decrease in bacterial adhesion to silicone and acrylic after pre-contact with both conditioning substances. The only exception was observed for strain IE186, which adhered at a similar extent to acrylic pre-contacted with gentian violet and non-pre-contacted acrylic.

Conclusion: The results obtained in this work have a potential clinical significance showing that both heparin and gentian violet are effective in reducing bacterial adhesion. Pre-treatment of biomedical materials with these substances may constitute a successful and expedite procedure to reduce the incidence of nosocomial infections, especially during the insertion of indwelling medical devices.

P1115 Transmission of influenza virus within a French university hospital during three influenza seasons, from October 2004 to April 2007

P. Vanhems, C. Regis, F. Najioullah, B. Barret, C. Gorain, S. Pires-Cronenberger, L. Pollisard, V. Escuret, F. Morfin, B. Comte, D. Floret, B. Lina (Lyon, FR)

Objectives: A prospective study was conducted during 3 influenza seasons at Edouard Herriot Hospital (Lyon, France) from October 2004 to April 2007 to describe nosocomial cases of influenza and to document the role of healthcare workers (HCW) in the transmission of this virus. This hospital counts for 1,000 beds.

Method: In 2004/05 (study #1) 15 wards participated to the study, 36 wards in 2005/06 (study #2) and 39 in 2006/07(study #3). HCW and hospitalised patients with Influenza Like Illness (ILI) syndrome were included. A nasal swab was taken for virological diagnosis. Medical history and vaccination status were collected for each case. Nosocomial cases were defined as occurring after at least 72 hours of hospitalisation for the patients or exposure to an infected colleague or a patient for HCW. Descriptive statistics were used for data reporting.

Results: A total of 872 ILI cases were identified, 71 in 2004/2005, 365 in 2005/2006 and 436 in 2006/2007. Children (Ch) were included only in studies #2 and #3. The sample counted for 168 adult patients (AP), 151 HCW and 553 Ch. There were 39 AP, 27 HCW and 90 Ch with laboratory confirmed influenza; 21 nosocomial influenza cases were observed; 13 for the AP, 5 for the HCW and 3 for the Ch. In study #1, 9 transmissions were observed from patient to patient (n = 3), from patient to HCW (n = 3) and from HCW to HCW (n = 3); in study #2 one transmission was observed from patient to HCW and in study #3, 11 transmissions were observed from patient to patient (n = 3), from patient to HCW (n = 3) and from HCW to HCW (n = 3). 2 patients with severe underlying disease and with confirmed hospital acquired influenza died. Among ILI cases 77/168 AP, 14/553 Ch and 47/151 HCW were vaccinated against influenza. 21 (15%) cases of confirmed influenza occurred in 138 vaccinated individuals (16 patients and 5 HCW). In study #1, circulating influenza A strain differed from the vaccine strain. Communautary influenza epidemic was moderate during study #2 and #3. In study #2 Influenza B was more prevalent and might be more easily transmitted than influenza A.

Conclusion: Nosocomial influenza occurred at each season which highlights the need of using appropriate preventive measures for control. Most transmissions were from non-vaccinated individuals, underlining the importance of vaccinating HCW to limit transmission.

P1116 How does vaccination of healthcare workers affect the number of influenza virus infections in nursing home patients?

C. van den Dool, E. Hak, J.C.M. Heijne, M.J.M. Bonten, J. Wallinga (Utrecht, NL)

Objectives: In epidemiological studies an increase in influenza vaccine uptake by institutional health care workers (HCWs) was associated with decreased influenza illness and mortality rates during the influenza season among nursing home patients. Since these studies were restricted to a small number of nursing homes in few influenza seasons with different vaccine-strain matching and vaccine coverage, the generalizability of these findings remains uncertain. We, therefore, developed a simulation model to determine the effects of increased influenza vaccine uptake by HCWs on influenza attack rates among nursing home patients in different scenario's and for different vaccine coverage levels.

Methods: We use a stochastic individual-based model with discrete time intervals to simulate influenza virus transmission in a 30-bed long-term care department. We track patients and staff and their stage in the influenza virus infection cycle: Susceptible, Exposed (infected but not yet infectious), Infectious and Recovered/immune. An individual's risk of being infected depends on the number of contacts it has, and the likelihood that the contacted persons are infectious.

Individual vaccination is assumed either to induce perfect immunity against infection or to have no impact at all. Most parameters in the model are based on earlier observations (e.g. latent and infectious periods, contact structures). The impact of uncertainty in some parameters (such as vaccine efficacy and transmission probability) was evaluated using a Latin Hypercube Sampling uncertainty analysis.

Results: Increasing vaccination of HCWs decreases the number of influenza virus infections in patients linearly; for every 25% of HCWs vaccinated, the patient attack rate decreases with 4%. No herd immunity is observed. The maximal relative risk reduction for patients (when HCW vaccination rate increases from 0% to 100%) is 67%. Increasing vaccination of HCWs decreases both the number of introductions of influenza virus into the patient population, and the total number of infections following an introduction. Variation in attack rate per department is high.

Conclusion: Increasing vaccination of HCWs can prevent a large fraction of influenza virus infections among nursing home patients and reduce the number of institutional outbreaks. The absolute risk reduction for patients is equal for every 10% increase in vaccine coverage among HCWs, independent of the initial coverage.

P1117 Enterobacteriaceae producing extended-spectrum β-lactamase: duration of carriage and implication for surveillance

C. Bellini, I. Nahimana, L. Senn, A. Wenger, P. Francioli, G. Zanetti (Lausanne, CH)

Background: In 2004–2005, ESBL-producing enterobacteriaceae (ESBL-E) were found in 1.2/1,000 patients in our hospital. ESBL-E were of CTX-M type in 79% of the cases (Lartigue MF et al, AAC 2007), and represented 0.7% of all enterobacteriaceae. In 2006, the rate of colonisation/infection by ESBL-E raised to 2.2/1,000 patients, representing 1.7% of all enterobacteriaceae. No outbreak was detected. This increase prompted more stringent infection control measures in addition to contact precautions, which included routine screening of ESBL-E carriage in all readmitted patients with a history of previous infection/colonisation by ESBL-E.

Objective: To describe the duration of ESBL-E carriage.

Setting: 850-bed general university Hospital.

Method: Duration of carriage was described in patients for whom follow-up screenings were available. These included i) readmitted patients with a history of ESBL-E, who were identified by an automated alert and underwent rectal swab and culture of any previously positive site; and ii) patients who had non-systematic serial screenings because of prolonged hospital stay.
Results: From April 2006 to November 2007, ESBL-E was detected in 91 new patients. Detection occurred <48 h after admission in 46/91 (51%); ESBL-E were *Escherichia coli* in 69 cases (76%) and *Klebsiella pneumoniae* in 15 (16%). 47/91 new patients had 1 to 9 (median 2) follow-up screenings in the context of either a prolonged hospital stay or a new admission. In addition, 9 patients with history of ESBL-E before the study period had 1 to 8 (median 2) follow-up screenings in the context of a new admission. In the total population of 56 patients with follow-up screenings, 38 (68%) had persisting colonisation after a median period of 56 days (range 1 to 608). The other 18 (32%) were eventually negative after a median period of 126 days (range 8 to 358).
Conclusion: prolonged carriage of ESBL-E is frequent, which warrants routine follow-up screening of all patients with a history of infection or colonisation by ESBL-E at the time of new hospital admission.

P1118 Biological monitoring of sterilisation procedures in dental offices in Venezuela

T. Bello, A. Lodato, C. Arango, C. Marrero, J.H. de Waard, L.A. Guevara (Caracas, VE)

Objective: To evaluate the incidence of sterilisation failures in a convenience sample of dental offices in Venezuela.
Methods: 284 strips with spores of *Geobacillus stearothermophilus* and *Bacillus atrophaeus* and an evaluation form were distributed to dentists interested in participating in this study. The dentists mailed the processed strips and the evaluation form to the laboratory for culture.
Results: Tests were submitted from 108 dental offices. The sterilisation methods were convection dry heat (93%) and steam under pressure (7%). A total of 32.4% sterilisation failures were detected. Convection dry heat failed with 32% (32 out 100) and steam sterilisation with 37.5% (3 out of 8)
Conclusion: A high percentage of failures occurred in the sterilisation of instruments reprocessed for the use in patient treatment. Steam under pressure failed more often than convection dry heat sterilisation. These results show the need for official guidelines to be developed by Venezuelan health authorities and to make it compulsory for dentists to biologically verify sterilisation processes. When corrective action is taken, routine use of spore strips for quality control could increases patient safety.

P1119 From "chicken run" to quality care and best practice in management of patients with infections caused by extended-spectrum β-lactamase-associated bacteria

A. Guleri, R. Palmer, B. Lunt, K. Lunt (Blackpool, UK)

Objectives: Recent ITV show and press news successfully drew the attention of public and doctors towards the overlooked rising problem in community with ESBL-producing strain of *E. coli*, associated mortality, association with consumption of foreign poultry and limited national surveillance. This media coverage did miss the complete picture. High quality patient care and best medical practice has been a top priority at Blackpool Victoria Hospital that witnessed rapid rise in ESBL associated infections especially community patients with UTI. This surveillance study over 2 $\frac{1}{2}$ years provides data on rising rate of ESBL infections in hospital and community, associated variations, susceptibility, and guides our strategy of optimal management of community and hospitalised patients.
Methods:

- Laboratory data on ESBL testing (since February 2005);
- Research: Comparing single plate double disk synergy technique with four combination disc technique plates for ESBL testing.
- Direct ESBL screening/confirmatory test on positive blood cultures.
- Standard comments on reports: multidrug resistance mechanism, infection control protocol and discussion with microbiologist in planning management.
- Risk stratification approach for severe sepsis in admission and emergency units.
- Enhanced liaison with GPs for patient specific discussion;
- Raising awareness.

Results: Rising rate of ESBL positive bacteria, especially *E. coli* seen in the last 2 $\frac{1}{2}$ years (refer graph). 619 isolates were confirmed ESBL positive. 34.5% (community specimens). Overall 70.1% (urines) and 10.5% (blood cultures). *E. coli* (62%), followed by *Klebsiella* sp. and *Enterobacter* sp. (approx 20% each). Overall susceptibility of *E. coli*: Ciprofloxacin, Gentamicin and Tazocin (16 – 50%). Urinary *E. coli*: nitrofurantoin (79%), trimethoprim (18%), ciprofloxacin (14%), gentamicin (71%), tazocin (46%). Susceptibility to imipenem 100%.
Conclusions: There has been a rapid emergence of CTX M-*E. coli* in England causing UTIs in community population. Blackpool is witnessing a similar phenomenon. Our comprehensive approach to the problem has included raising awareness in GPs, hospital consultants and junior doctors (especially from admission units), encouraging patient specific management discussion, regular local surveillance and feedback, and risk stratification approach for sepstic patients in admissions. Single daily dosing IV ertapenem is currently offered from assessment units and in near future to be administered at patients home.

P1120 Epidemiological typing of multi-resistant *Klebsiella pneumoniae* strains by FT-IR

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Klebsiella pneumoniae is an important nosocomial pathogen that has the potential to cause severe morbidity and mortality, not only in intensive care units and amongst paediatric patients, but also in medical and surgical wards.
Objectives: Evaluate the usefulness of a novel application of FT-IR spectroscopy in identification of multiresistant *Klebsiella pneumoniae* (MRKP) clones responsible for nosocomial infection outbreak in Portuguese hospital.
Methods: Seventeen multiresistant *Klebsiella pneumoniae* strains isolated from eleven patients from December 2006 to May 2007 during an outbreak in a Portuguese hospital were studied to identify MRKP clones by antibiotic susceptibility test using the disk diffusion method on Muller–Hinton agar (Kirby–Bauer methodology). For the antimicrobial agents tested inhibition zone diameters were measured and used only with the objective to identify and to compare the different clones. Simultaneously to the antibiotic susceptibility testing, strains were evaluated as well by Fourier transformed infrared spectroscopy (FT-IR – Bruker Optik GmbH). Several pre-processing methods have been investigated and the best treatment was selected based on its contribution for the chemometrics model robustness.
Results: As determined by disc-diffusion antibiotic susceptibility testing, MRKP isolates were distributed into to seven different antibiotypes groups. Two major clones responsible for the nosocomial infection were identified. Eight strains belonged to the same resistance pattern, designated by clone A. Another four strains belonged to another group that shows a different resistance pattern, designated by clone C. The other five strains have shown different resistance patterns while compared with each other as well as while compared with the previous clones. The FT-IR analysis of the same strains showed results concordant with the disc-diffusion antibiotic susceptibility testing and identified clone A and C as the predominant clones.
Conclusion: FT-IR is cheaper, easier and faster to perform and allow the access to the overall information of the bacterial cell. Our results demonstrate that application of FT-IR spectroscopy together with chemometrics methods has considerable potential to effectively fulfil the current requirements for rapid and correct differentiation of closely related strains implicated in nosocomial outbreaks.

P1121 Presence of ESBL-producing Enterobacteriaceae in the stools of outpatients with intra-abdominal infections requiring surgery

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Objective: Bowel flora may provide a substantial reservoir of resistant microorganisms and precedes intra-abdominal infections. Their susceptibility patterns may provide useful information for empiric treatment. We determinate the flora that colonises the bowel, the incidence of ESBL-producing and/or carbapenem-resistant Enterobacteriaceae (Eb) and the colonisation by non-fermenters Gram-negative bacilli (NF-BGN) in the stools of outpatients with intra-abdominal infections requiring surgery in 71 selected patients from 2005 to 2007.

Methods: Rectal swabs were collected at baseline and inoculated on MacConkey agar plates (MC), MC supplemented with cefotaxime (CTX) 1 mg/L, MC with ceftazidime (CAZ) 1 mg/l and in modified CCDA containing cefoperazone 32 mg/L. Bacterial colonies were identified from each plate using routine laboratory techniques. Eb growing in antibiotic supplemented media were identified and screened for ESBL production by the double-disk synergy test. Confirmation was performed by E-test with CTX/CTX-clavulanic acid (CA), CAZ/CAZ-CA and cefepime (FEP)/FEP-CA.

Results: Rectal swabs were obtained at baseline in 71 selected patients. In 69 of 71 patients (table) growed Eb and abscence of G– faecal flora in two patients. Nine (12.7%) patients harboured an ESBL-producing Eb (4 *E. coli* CTXR-CAZS-FEPS, 2 *E. coli* CTXR-CAZR-FEPS, 2 *E. coli* CTXR-CAZS-FEPR and 1 *C. freundii* CTXR-CAZS-FEPS) and 9 patients were colonised by NF-BGN (12.7%).

Table: Species of Enterobacteriaceae identified and susceptibility to some β-lactams

		Resistance (%)				Ertapenem	
	n/m	AMC	CTX	CAZ	FEP	MIC_{90}	range
E. coli	77/65	11.68%	14.28%	3.89%	3.89%	0.016	<0.002–0.094
K. pneumoniae	5/5	40%	0%	0%	0%	0.016	0.006–0.016
K. oxytoca	5/5	20%	0%	0%	0%	0.012	0.004–0.012
C. freundii	5/5	80%	40%	20%	0%	0.064	0.006–0.064
P. mirabilis	3/3	0%	0%	0%	0%	0.012	0.006–0.012
E. cloacae	5/4	100%	20%	20%	0%	0.19	0.008–0.19
H. alvei	2/2	100%	0%	0%	0%	0.32	0.016–0.32

n: number of isolates; m: number of patients; AMC: amoxicillin/CA.

P1122 Risk factors of hospital-acquired infections in a neurology-neurosurgery intensive care unit, in a tertiary care hospital, in Turkey

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Objectives: Intensive care unit (ICU)-acquired infections are associated with high mortality, excessive length of ICU and hospital stay, and high hospital costs. The risk factors for nosocomial infections may differ according to the type of ICU. The aim of this study was to evaluate the risk factors for ICU-acquired infections in the patients treated in neurology-neurosurgery ICU.

Method: The study was conducted in Ankara Training and Research Hospital, from May 2006 to May 2007. The patients treated for more than 48 hours in 14-bed neurology-neurosurgery ICU were enrolled into the study. The patients were followed until death or three days after discharge by prospective daily surveillance. Nosocomial infections were identified according to CDC criterias. Risk factors for ICU-acquired infections were analysed with a logistic regression model.

Results: Two hundred and fiftyfour ICU-acquired infections occurred in 160 (36.1%) of 443 patients during 5224 patient-days. The overall rate of ICU-acquired infection was 57.3/100 patients and 48.2/1000 patient-days. The most common site-specific infections were urinary tract infections (38.5%), pneumonia (31.7%), and bloodstream infections (22.9%). Urinary catheter-associated urinary tract infection rate was 23.7/1000 urinary catheter-days; central line-associated bloodstream infection rate was 37.9/1000 central line-days and ventilator-associated pneumonia rate was 31.6/1000 ventilator-days. The utilisation ratios of urinary catheter, central line catheter and ventilator were 0.90, 0.39 and 0.34, respectively.

In univariate analysis, age >60 years, being a neurology patient, presence of nasogastric tube, central venous catheter and arterial catheter, mechanical ventilation were determined as significant risk factors for ICU-acquired infections ($p < 0.05$). Multi-variate logistic regression analysis revealed, being a neurology patient ($p < 0.01$), presence of nasogastric tube ($p < 0.01$), presence of central venous catheter ($p < 0.01$), mechanical ventilation ($p < 0.1$) and parenteral nutrition ($p > 0.05$) as independent risk factors.

Conclusion: In this study, ICU-acquired infection rates were found higher when compared with NNIS results. In interpretation of device-associated infection rates, those high rates made us to take immediate precautions both for decreasing device utilisation and emphasising the importance of device application and care practices.

P1123 Screening cultures for multidrug-rresistant acinetobacter and MRSA in hospitalised patients

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Objectives: The aim of this study was to describe the microbiological performance of screening cultures (SC) for multiresistant *Acinetobacter* spp. (ACI) and meticillin resistant *Staphylococcus aureus* (MRSA) isolated from hospitalised patients.

Methods: Axillary (A), Faringeal (F), Nasal (N), and Rectal (R) swabs for carriers control of ACI and MRSA received from hospitalised adult patiens were studied during a year (October 2006 to September 2007). In the same period, prospective SC were performed in all Intensive Cure Unit (ICU) patients in the first 24 h of their admission.

Samples were processed by conventional methods, and also in an ACI selective medium (Leeds medium, Bio-Médics, Spain) and in MRSA-ID screening medium (bioMérieux). Identification and antibiotic susceptibility was performed by Wider System ® (Soria Melguizo, S.A., Spain).

Results: We processed 2544 samples (704 A, 963 F, 782 N, and 663 R). 480 of this were from ICU patients. These cultures represent the 11% of all bacteriological cultures received from hospitalised patients in the same period in our laboratory.

113 new patients were colonised or infected by ACI, and 65 of them were detected by SC. Rectal swab was the most useful (51% of 65 patients) followed by faringeal swab (38%). 74% of patients with ACI were from ICU and all but 2 were nosocomial.

In the same year we had 85 new pacients with MRSA, 29% of them were detected in SC. Nasal swab detected 60% of all isolates and faringeal swab 34%. We detected 2 patiens with MRSA at the time of their ICU admittance.

Conclusions: We point out the high work load that screening controls cultures represents for the microbiological laboratory.

The best sample for ACI detection was R, and N was for MRSA. In this setting we found Leeds medium very useful in rectal screening of ACI carriers.

SC were useful in detecting patiens colonised by multirresistant microorganisms in ICU patiens at the time of their admittance

P1124 Information overload or under load! How national knowledge weeks can help busy professionals

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The National electronic Library of Infection (NeLI) www.neli.org.uk is a single-entry portal to evidence based medical knowledge around infection and covers a broad range of infectious diseases.

The National Resource for Infection Control (NRIC) www.nric.org.uk was launched in May 2005 in response to National Audit Office (2000/04) recommendations for a national infection control manual. The project, funded by the Department of Health (UK), is endorsed by NeLI and provides a single access point to the best available evidence and resources on infection prevention and control.
The overall aim of both NeLI and NRIC is to provide relevant evidence based policy, guidance and quality information, published within the last 5 years (where possible) in a timely manner. The key added value of both sites is the quality appraisal of posted documents, which is conducted in collaboration with major professional societies and expert committees in the UK.
Promotion of the websites is ongoing through conference attendance and publications, but it is often obvious when talking to infectious disease and infection prevention specialists that they struggle to keep up-to-date with guidance, research and policy in this important area. Therefore, in January 2006 we started producing a monthly eNewsletter, which is currently subscribed to by more than 1800 professionals, and this provides information on the latest news, upcoming conferences and new resources added to the websites in the last month.
In an effort to help with either information 'overload' (too much information) or 'under load' (not knowing what you need to know) it was agreed that National Knowledge Weeks (NKW's) were the way forward. The aim was to identify the best resources, policy and expert comments regarding the prevention and/or control, treatment and care of specific diseases, infections and infection control practice for healthcare professionals.
This presentation outlines the three specialist areas promoted through the websites in 2007; Tuberculosis, Seasonal Influenza, and Infection Control along with the subsequent analysis of page views received, the popularity of the page, the geographical location of the incoming traffic and the means of accessing the page through use of the web server logs. The discussion will include an evaluation on whether NKW's add value to the sites and achieve their aim and represent the way forward for 2008.

P1125 Implementation of the "Do Bugs Need Drugs?" programme in British Columbia, Canada

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Objective: To implement a province-wide program for wise use of antibiotics in British Columbia (BC), Canada (population 4.3 million).
Methods: Educational resources for healthcare providers, the public and children were adopted directly or adapted from the Do Bugs Need Drugs? (DBND) program, which originated in 1998 in the adjacent province of Alberta. DBND key messages promote: 1) handwashing prevents infections, 2) bacteria and viruses are different, and 3) use antibiotics wisely.
In 2004, based on the success of DBND in Alberta, the British Columbia Ministry of Health provided $1.4 million to implement the program in BC. A program coordinator and assistant were hired. A steering committee with representatives from all health regions was organised as was a DBND working group. An extensive training program was developed with nursing students to teach Grade 2 (7–8 year olds) and daycare children. A 20 page parent guide was distributed to all public health units. A mail out of print materials and an antimicrobial reference guide was distributed to all physicians, dentists and pharmacists in the province. A provincial media campaign included advertisements on television, in movie theatres, parent magazine, and public transportation stations, buses and trains. Collaborations were established to follow prescribing rates and resistance patterns over time. An initiative with a food/pharmacy chain has been established for pharmacy personnel to deliver the program in communities throughout the province.
Results: Since 2004, educational materials have been distributed to 8665 physicians, 1078 trainees, 3795 pharmacists and 2865 dentists. Continuing education sessions have been provided to 863 physicians, 175 pharmacists, 210 infection control practitioners, 400 nurses and 520 nursing students. The Grade 2 program has targeted 12,950 children (>40% of Grade 2 children in Vancouver) and the daycare program has targeted 3384 children, 441 care providers and 39 childhood education students. The televison advertisement has reached 75–85% of the target audience (females aged 25–54) an average of 6–12 times over a 4 week period.
Conclusions: Recognising that health promotion campaigns have a significant delay between education and outcomes (in this case, reduced rates of antibiotic resistance), the BC government has committed an additional $2 million over the next three years.

P1126 Improving outbreak management by understanding barriers faced by professionals in hospitals and public health

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Objectives: The effectiveness of outbreak control depends substantially on the adherence to guidelines. Adherence to guidelines is often not optimal and this has been related, among others, to knowledge, attitudes and behaviour of professionals. Little is known about the factors that influence adherence to authoritative guidance in emergency situations. This study aimed at identifying the most important constraints and facilitators related to professionals in order to improve the quality of outbreak management.
Methods: To qualitatively study and understand barriers to appropriate outbreak control, 26 in-depth interviews with hospital microbiologists and infection control professional and public health physicians and nurseswere carried out in the Netherlands. The conceptual framework of Cabana (JAMA, 1999) was used to discuss recommendations on case finding, infection control, diagnosis, treatment and prophylaxis taking into account recent crisis situations (SARS, outbreaks of *C. difficile* ribotype 027, rubella outbreak). After classifying the barriers, questionnaires were developed for each group of professionals including: 38 items for public health physicians, 40 items for microbiologists, 26 items for infection control professionals and 40 items for public health nurses. The questionnaires were subsequently administered to hygienists in hospitals (N= 95), physicians and nurses in public health services (N=33) and a representative sample of microbiologists (N=30). A five point, asymmetric Likert scale was used to assess appropriateness of each item.
Results: The response amounts 96% for the public health physicians, 84% for the public health nurses, 63% for the hygienists. The study is still ongoing among microbiologists. Preliminary results show that adherence increases when outbreak control measures are evidence based, do not disturb the daily routine in the hospital/department and take into account the safety of the personnel. Effective communication between microbiologists and clinical specialists increases the adherence to infection control measures and therapy recommendations.
Conclusions: The quality (evidence based development, applicability) and timeliness of scientific guidance during emergency situations are among the most important predictors for the reported adherence by professionals. Further qualitative and quantitative analysis of the data will provide a comprehensive basis for tailored interventions to improve adherence.

P1127 Compliance with contact precautions in acute care teaching hospital

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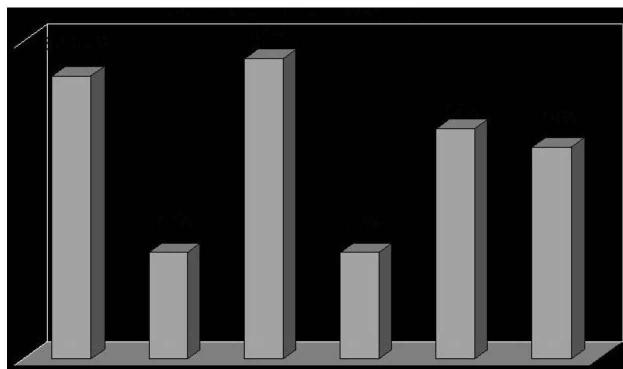
Introduction: The essential factors for limiting the spread of Meticillin resistant *Staphylococcus aureus* (MRSA) are the surveillance cultures of MRSA infection or colonisation in high risk group of patients admitted to the hospital in addition to the use of contact precautions. On the other hand, poor adherence to contact precautions and poor hand hygiene practices among healthcare workers (HCWs) are among the main reasons for the nosocomial spread of MRSA and failure of the infection control programs.

Methods: Between May 2nd and Jun 5, 2005 we conducted an observational study to monitor the overall compliance with the contact precautions with regard to MRSA among doctors, nurses, medical students and other HCWs during routine adult patient care. These precautions included: hand washing; usage of gloves, gowns and mask, and cleaning equipments after usage. This observational study was conducted in the setting of acute healthcare teaching hospital. The observation was performed in 10 hospital inpatient units which had patients who were colonised or infected with MRSA.
Results: We observed more than 455 opportunity of compliance to the ICPs. 130 opportunities were observed among males and 325 among females. These observations were as follows: among doctors were 94; nurses were 199, among medical students were 50 and among other HCWs were 114 observations. The overall compliance rate of all HCWs to the ICPs was 67%. Compliance observed among males was significantly less than among females, 73% vs 80% (P= 0.05). The overall compliance rate to hand washing was 77% (fig. 1).



There was no significant difference in hand washing compliance rate between doctors and nurses 82% vs 83% respectively (p= 0.9). However, the observed nurses were statistically more compliant to put on gowns (p = 0.02), gloves (p = 0.02) and masks (p = 0.01) than doctors. The rate of cleaning equipments when removed from the patient room by any HCW was only 25%. Eighty five percent of the observed HCWs preferred to use alcohol-based dispensers than the soap and water.
Conclusion: Unlike other investigators, we found that nurses washed their hands not more often than did doctors but both groups washed there hands more often than medical students and other healthcare workers. This is because nurses and doctors had the most opportunities (82% & 83%) to wash their hands.

P1128 Analytical study of infection control measures in Russian ICUs: lessons from real life

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Introduction and Purpose: Epidemiological assumptions show that 2–2.5 millions of patients acquire healthcare associated infections (HAI) in Russia annually. To uncover reasons for the high prevalence we aimed to assess common practices of infection control (IC) in Russian ICUs.
Methods: The study was conducted in 38 multidisciplinary (MD) ICUs from April to August 2007. Project coordinators worked in each of 23 cities and distributed specific questionnaires among ICUs medical doctors. The data were than collected and analysed in reference centre of the institute of Antimicrobial Chemotherapy of Smolensk State medical Academy.
Results: Out of 400 ICU physicians' questionnaires 84.5% were from MD and 15.0% from specialised hospitals (SH). No information was provided in 0.5%. Absence of single patient rooms for isolation was noted in 57.5%; presence of one single room was indicated in 12.8%, 2 – in 20.3%, 3 and over – in 9.1% of respondents' answers. Duration of stay in MD hospitals varied from 1.9 to 18.0, in SH – from 1.9 to 36.0 (average 4.5 days). Availability of liquid soap and/or alcohol hand rub doser in wards was registered in 74.8% of respondents' answers. Use of individual alcohol hand rub solutions after contact with patient reported 62.8% of respondents. Presence of HAI guidelines on antimicrobial chemotherapy was registered by 51.8% respondents only. Compliance with guidelines averaged 82.3%. No guidelines for insertion and catheter care reported 10.8% respondents. Routine MRSA carriage screening on admission to MDH was registered in 17.8% of respondents and in 26.7% for SH; routine use of mupirocin for MRSA decolonisation was registered in 3.6% of respondents in MDH and in SH – in 38.3%.
Discussion: Official statistics indicates prevalence of 30,000 of HAI in Russia annually. This was one of the very first studies to assess real situation with IC that found comparatively low compliance with existing measures in ICUs in combination with virtual absence of quintessential barrier measures, use of alcohol hand rub solutions and adherence to guidelines.

P1129 European survey and consensus on infection control standards and performance indicators

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Objectives: To assess the situation in European Member States (MS) regarding the progammes, structures and processes in place for HCAI and AMR stewardship and whether there was a consensus amongst infection control professionals and leading healthcare workers for proposed standards and performance indicators (SPIs).
Methods: A questionnaire survey was performed in all MS to explore the current situation. A steering group (Andreas Voss, Peet Tull, and Carl Suetens) helped inform the project group as to the design of national and local SPIs, which were then to be explored in detail, as well as practicality and level of detail. We used the results of several EU consensus meetings and DG SANCO documents on HCAI/AMR that had been explored with MS's departments of health. Data were analysed within Excel(TM) spreadsheets.
Results: 29 (88%) of the 33 MS replied. Analyses showed that 72% had a national HCAI, and 62% an AMR, programme. National laws were in place (55% HCAI/32% AMR). Reductions in HCAI/AMR were targets for approximately two-thirds of programmes, but much variation in responsibilities (9 explored) and topics covered (9 explored) e.g. only 12 MS produced an annual report and 14 covered aspects of occupational health. The same was true for PIs (7 explored), e.g. only 5 considered hand hygiene. Training was considered in only 43% for HCAI and 71% for AMR. HCAI surveillance (78%: 59% compulsory, 41% recommended) and AMR (95%: 55%, 45%). The results clearly indicated a wide variation and the need for a consensus. National and local SPIs were developed: five groups with 51 SPIs. 82% (28/34) MS responded: an average of 83% agreed with these, 12% agreed with alteration, 2% were neutral and 3% disagreed. There were 138 statements of underlying principles relating to these SPIs (82% agreed; 6% with alterations, 5% neutral, 3% disagreed. 89% thought the tools were practical, 57% thought the level of detail was about right but 39% thought it was too much.
Conclusions: We have described a wide variation in SPIs in Europe and proposed SPIs that had a high level of agreement with relevant healthcare workers. Further discussions will need to take place within the project and with MS to explore issues of practicality.

P1130 Optimising the flow of ID-AST information between microbiology laboratory and intensive care unit with the new feature of EpiCenter version 5.5 connected to the BD Phoenix system

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Objectives: Rapid ID and AST can have a significant impact on the management of infections especially with the spread of multidrug resistant bacteria to the community. Recently released systems that perform automated ID and AST, benefiting from advanced expert systems, can impact the workflow of the microbiology laboratory for a typical hospital. The connection to the hospital information network enhances the transmission of microbiological results from the lab to

the primary care physician, thus potentially affecting the management of the patient. Whether this direct link improves patient's care is still questioned. In this pilot study, we compared the transmission by courier of paper printout results from the laboratory to the ICU to that using the new feature of EpiCenter version 5.5 ID-AST system (BD Diagnostic Systems, USA).
Methods: During a period of 15 days, we measured times to effective transmission, from Gram stain, culture, ID-AST to final validation (internal turn-around time [TAT]), from final validation to ICU, and from ICU to the prescriber (external TAT). EpiCenter version 5.5 connected to the Phoenix ID-AST system was not connected to the laboratory informatic software during this study.
Results: Internal mean and median TAT from preliminary results to validation was 1 h47mn and 30mn respectively [0–19 h15mn] and internal mean and median TAT from ID and AST results to validation was 2 h42mn and 2 h respectively [30mn-6 h30]. Internal TAT by EpiCenter was 0mn, as the system together with the Phoenix ID-AST automate allowed electronic networked transmission after validation.
By courier, median and mean TAT for preliminary results and/or ID-AST results only were 25 h10mn and 55 h55mn respectively. By comparison, median and mean TAT with the use of EpiCenter were 21 h36mn and 25 h11mn respectively.
When considering the time between specimens' arrival in the laboratory and the final results' transmission to the ICU and to the prescriber, external mean and median TAT from validation to ICU by courier was 3days-21 h-31mn and 3days-8 h-47mn respectively. By comparison, median and mean external TAT with the use of EpiCenter were 1day-19 h-55mn and 1day-8 h-18mn; which correspond to a 55% time reduction.
Conclusion: We observed a 55% internal and external mean TAT reduction. This encouraging result will allow us to set up a protocol investigating benefits in clinical outcome provided by real-time ID-AST results transmission.

Infections in the immunocompromised host

P1131 Pneumonia in patients with cancer

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Objectives: The epidemiology of infections in cancer patients undergoes periodic changes which impact empiric therapy. Pneumonia is the most common site of infection in such patients. Our primary objective was to describe the current microbiology of pneumonia in cancer patients presenting to our emergency centre (EC).
Methods: Retrospective review of medical records over a 5 month period in 2006.
Results: A total of 7,784 EC visits of which 272 (3.5%) were due to episodes of pneumonia, occurred. Of these, 98 (36%) had positive cultures from respiratory specimens and/or blood. Fifty-seven (58%) had an underlying hematologic malignancy (HM) whereas 41 (42%) had an underlying solid organ cancer (SOC). Respiratory specimen cultures were positive in 65 patients (66%), blood cultures only in 21 patients, and both blood and respiratory cultures in 12 (12%). Sixty-one patients (62%) had monomicrobial infections, whereas 37 had polymicrobial infections. The proportion of polymicrobial infections was higher in patients with SOC (49%) than in patients with HM (30%). The organisms isolated most often from respiratory specimens of patients with SOC were *Pseudomonas* spp., *Stenotrophomonas maltophilia*, various *Streptococcus* spp. and *Staphylococcus aureus*. A similar pattern was seen in patients with HM. Blood culture isolates were often different from respiratory specimen isolates when both sites were culture positive. *Candida* spp. but no moulds were isolated from patients with SOC, whereas moulds (mainly *Aspergillus* spp.) were isolated from patients with HM. Two mycobacterial infections and one case of PCP were also documented.
Summary and Conclusions: Pneumonia accounts for approximately 3.5% of EC visits at our Comprehensive Cancer Center. Thirty-six percent of these episodes are microbiologically documented. Organisms that cause community acquired pneumonia in other patient populations (*Streptococcus pneumoniae*, *Haemophilus influenzae*, *Legionella* spp., *Mycoplasma pneumoniae*) are seldom seen in cancer patients. Consequently, empiric therapy for cancer patients presenting with pneumonia may vary considerably from options mentioned in various guidelines. Ideally, empiric therapy should be based on local microbiologic and susceptibility data.

P1132 Detection of *Helicobacter pylori* DNA in liver tissue samples from patients with hepatocellular carcinoma

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Introduction: *Helicobacter pylori* (*H. pylori*) is one of the most common bacteria worldwide found in more than 50% of human population. The presence of *H. pylori* is the main cause of several gastro-duodenal diseases, including peptic ulcer and gastric cancer. It has been designated as class I carcinogen by the World Health Organisation. Chronic *H. pylori* is related to a variety of extra gastric diseases, including liver diseases.
Objectives: the aim of the present study is to evaluate the presence of *H. pylori* in human hepatocellular carcinoma (HCC) to determine if *H. pylori* may contribute to the development of this disease.
Materials and Methods: Liver specimens from 33 patients with HCC diagnosed by histopathology were studied. Tumour tissue samples were examined by polymerase chain reaction (PCR) for the presence of genomic 16S rRNA of *Helicobacter* genus using specific primers. Besides, other genes (26kDa cell surface protein, cagA, vacA) specific for *H. pylori* were also screened by PCR. The specimens were examined for *H. pylori* by immunohistochemical procedure using anti-*H. pylori* antibody.
Results: Genomic sequence of *H. pylori* was found in 11 of 33 (33.3%) tumour tissues. On the other side, *H. pylori* was proved to be immunohistochemically positive in 7 tumour tissues (21.2%).
Conclusion: The presence of *H. pylori* in HCC tissue by means of molecular methods and immunohistochemistry supports the concept of the close association between *H. pylori* and HCC development. But, their eventual role in hepatocarcinogenesis, although it is plausible, remains to be proven.

P1133 SeptiFast PCR combined with blood cultures for the microbiological documentation of fever in neutropenic cancer patients

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Background: Blood cultures (BC) identify the aetiology in only 1/3 of febrile neutropenic episodes. New diagnostic tools are thus needed for guiding investigations and antimicrobial therapy. LightCycler SeptiFast (Roche) is a real-time PCR for the detection of bacterial (9 G+ and 10 G– species) and fungal DNA (6 species) in blood.
Objective: To assess the utility of SF combined with BC for the microbiological documentation of fever in neutropenic cancer patients.
Material and Methods: Blood samples for BC (4 aerobic + anaerobic sets, Bactec 9140) and SF (4 EDTA tubes) were drawn simultaneously in adult febrile neutropenic cancer patients undergoing myeloablative chemotherapy for haematological malignancies. Microbiological documentation by SF and BC were analysed.
Results: 186 samplings (SF/BC 1:1) were analysed in 119 febrile episodes. 18/186 (9.6%) SF analyses were invalid (contamination of negative control), 12 (6.4%) were inhibited. 88 pathogens were detected in 59/119 (50%) febrile episodes by BC and/or SF (see table).
Using BC as reference, sensitivity of SF was 50%, specificity 64%, PPV 29%, NPV 81% and efficiency 60%. BC were positive in 20/74 (27%) and SF in 14/74 (19%) samplings drawn in the absence of antibiotic therapy. For samplings drawn during antibacterial therapy, BC were positive in only 12/112 (11%), whereas SF detected pathogens in 38/112 (34%, $p < 0.001$).

Conclusions: Septifast combined with blood cultures is a promising tool for the microbiological documentation in febrile neutropenic cancer patients.

	BC and SF (n = 14)	BC only (n = 25)	SF only (n = 49)
Gram-positives	3	18	11
Staphylococci	2	5	7
Streptococci	–	6	–
Enterococci	1	1	4
Other (not in SF panel)	–	6	–
Gram-negatives	11	7	29
Enterobacteria	7	5	8
Non-fermentative bacteria	4	–	21
Other (not in SF panel)	–	2	–
Fungi	0	0	9
Candida sp.			7
Aspergillus sp.			2

P1134 Quantitative CMV PCR in stem cell transplant patients: DNA manual extraction versus automatic extraction

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Introduction: The success of preemptive treatment of CMV infection in stem cell transplant (SCT) recipients before disease progress depends on efficient early diagnostic tests. The aim of this study was to compare a commercial quantitative CMV-PCR assay with DNA automatic extraction (TNAI-PCR) versus manual extraction (CA-PCR) in the diagnosis of CMV infection in our allogeneic STC recipients, comparing the results obtained by PCR with those obtained by antigenaemia (pp65).
Methods: All patients were prospectively monitored post-SCT with antigenaemia (pp65) CINApool® (Argene) and Cobas Amplicor CMV Monitor Test® (Roche) (DNA manual extraction, CA-PCR). A total of 211 blood samples obtained between January 2005 and May 2006 corresponding to 45 CMV infection episodes from 24 SCT patients, were tested retrospectively using COBAS Ampliprep TNAI kit® (Roche) (DNA automatic extraction system, TNAI-PCR).
An episode is defined since the first positive sample by any method until the first negative sample by both techniques. A positive sample was defined by antigenaemia $\geqslant$2 cel/4$\times$10^5 cel and/or PCR $\geqslant$600 copies/mL. Repeated positive samples were considered the same episode. Independent episodes in one individual were considered if there were at least 15 days of negative serial detections. Samples and episodes were analysed independently. Concordance between methods was determined by Kappa and Spearman coefficients. P-values lower than 0.05 were considered statistically significant.
Results: Good correlation was found between CA-PCR and TNAI-PCR (Spearman coefficient=0.8, $p<0.001$). Assuming qualitative interpretation of PCR, good concordance was also found between CA-PCR and TNAI-PCR (Kappa index=0.6, $p<0.001$).
Episodes analysis: As a whole, TNAI-PCR detected 39 out off 45 episodes (86.7%) and by CA-PCR 32 out off 45 episodes (71.1%) ($p=0.07$).
Twenty out off 45 episodes (44.4%) were detected by both techniques at the same time. Five episodes (11.1%) were detected only by antigenaemia, 8 episodes (17.8%) by TNAI-PCR only and 1 episode (2.2%) by CA-PCR.
In 24.4% episodes, TNAI-PCR was earlier than CA-PCR (median = 34 days). Thus in 42.2% (24.4%+17.8%) episodes TNAI-PCR improved CMV detection versus CA-PCR.
Conclusion: Automatic extraction improves sensibility detecting positive samples and lowers CMV infection diagnosis delay time in SCT patients. But in this moment, pp65 antigen detection cannot be replaced by all biological molecular methods.

P1135 Atypical mycobacterial infection in an allogeneic haematopoietic stem cell transplant patient with bronchiolitis obliterans

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Objectives: A 29 years old female with a previous diagnosis of chronic myelogenous leukaemia who had an allogeneic stem cell transplant (ASCT)in 2004 was admitted to the hospital with dyspnea and increased sputum production. After ASCT she had received cyclosporin A for GVHD and at the 9th month of transplantation had a diagnosis of bronchiolitis obliterans (BO). Steroids were started and continued. The course was complicated with lower respiratory system infections that were succesfully treated. 1.5 year after the diagnosis of BO, she was admitted for cough and progressive dyspnea. Chest X-ray revealed a heterogenous infiltration with a cavity in the left lung. Clinical and radiological findings were compatible with pulmonary tuberculosis (TB). Sputum, brochoalveolar lavage TB cultures with Bactec system and ARB were negative. Anti-TB therapy was initiated. There was no improvement and she returned back to the hospital 5 months later reporting that she did not use the drugs because of nausea and vomiting. There was progression on the pulmonary X-ray. Sputum was positive for ARB. She was transferred to the ICU two weeks later because of severe hypercapnia and respiratory failure. She was intubated and ventilated.
Methods and Results: The growth obtained in Bactec system was identified by PCR-RFLP as *Mycobacterium abscessus*. She received imipenem-cilastatin, amikacin and clarythromycin. In one month she improved clinically and pulmonary X-ray findings resolved. One month later, she was again admitted to the ICU because of hypercapnia and acidosis. She was intubated and mechanically ventilated. She had a diagnosis of ventilator associated pneumonia. She died one month after the second ICU admission. *Mycobacterium abscessus* is a very rare pathogen, a rapid grower, and causes disseminated infections in patients with cystic fibrosis, bronchiectasis, fibrosis due to previous mycobacterial diseases, immunosuppressed subjects and in lung transplant recipients. The agent has a tropism for the respiratory tree, produces tuberculoid granulomas with caseous lesions, and resistant to standard anti-TB drugs and is generally susceptible only to parenteral antibiotics and oral macrolides.
Conclusion: ASCT is a complex immunosuppressive state that may predispose to infections caused by any rare pathogen. We think this is the first case with *M. abscessus* infection after ASCT and the structural lung disease (bronchiolitis obliterans) caused predisposition to this infection.

P1136 Bloodstream infections after solid-organ transplantation

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Objectives: Analyzing the characteristics of bloodstream infections occurring in solid organ transplantation (SOT) recipients.
Methods: All bacteraemia among SOT recipients in our institution during the period from January 2002 to December 2007 were included in the study. We performed a retrospective chart review to determine the patient data such as age, gender, the duration from transplantation to a diagnosis of BSI, whether the BSI was hospital- or community-acquired. The primary BSI was defined as the bacterium isolated from blood culture, which is not related to an infection at another site. Otherwise it was defined as secondary BSI.
Results: A total number of 58 BSI episodes in 42 patients were diagnosed. Of these patients 11 (26.2%) were female. The ages of the patients were in the range 18 to 68 years, with a mean value of 42.1. There were 30 liver, 11 kidney, and one liver-kidney recipients. Of the episodes 17 (in 13 liver and 3 kidney recipients) were diagnosed within the first month after the transplantation, 22 (in 10 liver and 2 kidney

recipients) during the 1–6 months period, and 19 (in 7 liver, 6 kidney, and one liver-kidney recipients) after the sixth month. Of the episodes 10 were primary. The most common focus of BSI was abdominal among liver recipients with 35 (97.2%) episodes, whereas it was urinary among kidney recipients with 6 (54.5%) episodes. *Escherichia coli* was the most frequent agent in both groups of recipients. Two episodes were polybacterial. The list of causative agents was shown in the Table.

Table: Frequencies of causative agents of BSI among SOT recipients

Causative agents	Liver recipients	Renal recipients	Liver & renal recipients
Escherichia coli	21	5	–
Nonfermentative Gram-negative Bacilli	7	2	1
Enterococcus spp.	5	–	–
Staphylococcus aureus	4	–	–
Klebsiella pneumoniae	2	2	–
Streptococcus anginosus	1	–	–
Salmonella group D	–	1	–
Enterobacter spp.	2	2	–
Proteus spp.	1	–	–
Klebsiella pneumoniae & *Enterococcus* spp.	1	–	–
Klebsiella pneumoniae & *Morganella morganii*	1	–	–
Total (n = 58)	**45**	**12**	**1**

Conclusion: The number of BSI episodes was highest in the period of 1 to 6 months after the SOT. Secondary BSIs were considerably more frequent than the primary BSIs. The leading foci of secondary BSIs were consistent with the region of transplantation; that is abdominal for liver, whereas urinary for kidney SOT.

P1137 Infections in patients with myelodysplastic syndromes

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Objectives: To describe clinical characteristics and outcome of infections in patients with myelodysplastic syndromes (MDS).
Patients and Methods: Infections occurring in patients with MDS, cared for at the University Hospital of Heraklion, Crete, Greece from 2004 to 2007 were retrospectively reviewed.
Results: Fifty four episodes of infection occurring in 32 patients have been studied. The median age of patients was 77 years (range 66–95); 24 (75%) were males. At the time of infection, 9 patients (28%) had been classified as RAEB-I, 7 (22%) as RA, 7 (22%) as RAEB-II, 4 (12.5%) as RCMD, while 3 (9%) and 2 (6%) as RARS and 5q(−) syndrome, respectively. The median length of hospital stay was 9.6 days (range 2–50), while the median white blood cell count on admission was 1700 cells/μl (range 100–5500/μ). The most common was respiratory tract infection [27 episodes; (50%)], followed by urinary tract infection [7; (13%)]. Nine episodes were complicated by bacteraemia. The responsible microorganisms were identified in 19 (35%) of the episodes. The most frequently identified causative organisms were *Staphylococcus epidermidis*, isolated from 4 specimens (21%), *Enterococcus faecalis* from 4 (21%) and meticillin-resistant *Staphylococcus aureus* from 3 (16%). Regarding outcome, patients were classified into 2 groups: those with curable and those with complicated episodes. An episode was defined as complicated if responsible for the patient's death or prolonged hospitalisation (⩾14 days). There were 25 complicated episodes (46%) including 4 deaths (7%). The rest 29 episodes (54%) were curable. Univariate analysis showed a trend of patients with RAEB-I for complicated course as compared to patients with other MDS subtypes (P = 0.051), while patients with grade IV neutropenia (neutrophils <500/μl) developed infections significantly more frequently (P < 0.05). Multivariate logistic regression analysis revealed that grade IV neutropenia is associated with complicated course (OR: 8.306 95% CI: 1.286–51.235, P = 0.026).
Conclusions: Severe infections represent a serious threat for patients with MDS, especially those with grade IV neutropenia. Neutropenia and probably the MDS subtype may predict the relative risk.

P1138 Current trend in the epidemiology of bloodstream infections in patients with haematological malignancies and solid neoplasms in Iasi, Romania

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Objectives: to characterise the epidemiological and microbiological spectrum of systemic infections in hematological and oncological patients.
Methods: a prospective study of bloodstream infections was conducted between May 2004-June 2006 in two hospitals from Iasi.
Results: We investigated a total of 92 suspected episodes of systemic infections that occurred among 79 selected patients, involving 40 males and 39 females (mean age = 26 years old). There were 45 (48.91%) febrile episodes with positive blood cultures and 47 (51.09%) with negative blood culture. The most common microorganisms were Gram-positive cocci 15 (33.33%), followed by stable cell-wall deficient forms 9 (20.00%), Gram-negative bacili 9 (20.00%), Gram-positive bacili 5 (11.11%), fungi 5 (11.11%), and anaerobes 2 (4.44%). From the total 23 strains of cell-wall deficient forms (51.11%), 14 returned to their typical morphology (6 Gram-positive cocci, 5 Gram-positive bacilli, 3 Gram-negative bacilli) and 9 were L-stable.
Conclusions: (1) The isolation rate, 48.91%, was in accordance with literature reports; similar percentages of Gram-positive and Gram-negative isolates were found (the shift from predominating Gram-negative to a Gram-positive aetiology was noted). (2) We found a high proportion of cell wall deficient forms, rarely reported before. (3) Studies reporting local microbiological findings are necessary because they support an initial empiric antibiotic regimens for prophylaxies or therapy more accurately than from other areas. The right treatment protocols remain to be established.

P1139 *Strongyloides stercoralis* hyperinfection in an immunosuppressed patient with a nephrotic syndrome

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Objectives: Strongyloidiasis is highly prevalent in tropical and subtropical regions, with endemic foci in some temperate areas. Its real prevalence is probably underestimated.
We intend to alert to nephrotic syndrome as an unusual presentation of chronic infection, to the risk of potentially fatal hyperinfection syndrome in the immunocompromised population and, therefore, the need for a high suspicion and accurate laboratory diagnosis.
Methods: We report the case of a 31-year old Cape Verdean woman living in Portugal, admitted with profuse diarrhoea, abdominal pain and dehydration. She had been diagnosed with nephrotic syndrome (minimal change disease) of unknown aetiology four months before and was receiving prednisone and mycophenolate mofetil.
Bacteriological and parasitological investigation included examination of faeces, sputum, urine, blood and cerebrospinal fluid.
Results: A diagnosis of *Klebsiella pneumoniae* sepsis was made and *Strongyloides stercoralis* larvae were found in faeces and sputum. Hyperinfection was considered and mycophenolate mofetil was suspended, steroids tapered and albendazole was started. Eosinophilia on peripheral blood only became evident after suspension of the immunosuppressors (reaching 5.0×10^9/L). Human Immunodeficiency Virus and Human T-limphotropic Virus serologies were negative.
She was treated for a month, with dramatic clinical improvement, regression of the eosinophilia, absence of larvae on stool or sputum and resolution of the nephrotic syndrome (proteinuria was 13 g/day prior to therapy to 1.3 g/day on discharge).
Conclusions: Chronic strongyloidiasis is a rare and reversible cause of nephrotic syndrome.

Immunosuppressive therapy has a high risk of causing fatal hyperinfection or dissemination of *S. stercoralis* and, therefore, screening is advised, in patients with a sugestive epidemiological history, prior to its initiation. Combination of parasitological examination (including faecal concentration and culture) with serology is probably the best approach. The diagnosis of *S. stercoralis* hyperinfection should be considered in immunosuppressed patients from endemic areas presenting with acute respiratory or gastrointestinal symptoms and Gram-negative sepsis, even in the absence of eosinophilia. In these cases other body fluids should also be examined and attention should be payed, on routine examination, to the incidental finding of larvae.

P1140 Molecular evidence of a nosocomial *Pneumocystis jirovecii* transmission among 16 patients after kidney transplantation

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Objectives: In recent years, nosocomial clusters of *Pneumocystis jirovecii* (formerly P. carinii) pneumonia (PCP) among immunocompromised individuals were reported. Mostly, the source of infections was suspected within the clinical settings when transplant recipients and PCP patients shared hospital facilities.

Methods: We report a cluster of 16 renal transplant recipients positive for *P. jirovecii*. None of them received anti-*Pneumocystis* prophylaxis prior to *P. jirovecii* detection. Epidemiological studies revealed that 15 of them were kidney transplanted at a German university hospital and attended the same inpatient and outpatient clinic from January through September, 2006. Multilocus sequence typing (MLST) was performed on the following genes: ITS1, beta-tub, 26S and mt26S.

Results: *P. jirovecii* DNA was available from 14 patients and showed identical MLST types among these renal transplant recipients. Surprisingly, one patient who was treated at a different nephrological centre and negated personal contacts to patients from the renal transplantation cluster harboured the same *P. jirovecii* MLST type. Three HIV patients and one bone-marrow transplanted hematologic malignancy patient – treated at two different medical centres – were used as controls and revealed different MLST types. Interestingly, in three of the four previously described regions new alleles were detected and one new polymorphism was observed in the mt26S region.

Conclusion: The epidemiological data and the genotyping results strongly suggest a nosocomial patient-to-patient transmission of *P. jirovecii* as the predominant transmission route.

P1141 Critical care-related infections in heart transplant recipients

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Objective: Reports on infections of heart transplant (HT) recipients during early postoperative ICU stay (ICU-E) or requiring later ICU readmission (ICU-L) are very scarce. Our aim is to describe these infections in HT before and after use of daclizumab.

Methods: We reviewed ICU infections in our HT cohort (1989 to 2007) and compared ICU-E and ICU-L infections before and after daclizumab (1989–2002 vs 2003–2007).

Results: There were 48 ICU infections in 36/387 patients (9.3%): 25 (52%) ICU-E and 23 (48%) ICU-L.

ICU-E infections: Low respiratory tract infections (11; 44%), catheter-related bloodstream infections (BSI) (6; 24%), primary bacteraemia (2; 8%), mediastinitis (4;16%), gastrointestinal (GI) infections (1; 4%) and other (1;4%). Microorganisms were Gram(–) (12/48%), Gram(+) (9/36%), *Aspergillus* (2/8%), *Candida*, and *C. difficile* (1;4% each). All mediastinitis, 53.8% of the BSI, 45.8% of the pneumonias, and only 17% of the septic shocks occurred in the ICU-E stay. Mortality was 35% (7/25).

Median time to ICU readmission was 87 days (IQR:51–1037) and 52% of the infections were nosocomial. ICU-L infections were pneumonia (13/23; 56.5%; only 2 ventilator-associated pneumonia), disseminated infections (4; 17%), GI CMV infections (2; 8.6%), and others (4; 17%). All CMV infections (5/22%) and 4/6 aspergillosis occurred as ICU-L readmissions. Gram(–) (9; 39%) and Gram(+) bacteria (3; 13%) were also detected. Attributable mortality was much higher (13; 56%).

When pre- and post-daclizumab periods were compared, we detected a disappearance of all opportunistic infections (CMV, *Aspergillus*, *Pneumocystis*) requiring ICU admission in the post daclizumab period. From 2002, mortality per episode decreased (19/35 vs 1/13), as did the need for ICU readmission due to infection (1.6 vs 0.4 episodes/year).

Conclusion: 9% of HT recipients will have an infection during their ICU stay (52% in the first post-HT ICU admission). Opportunistic infections, septic shock, and BSI are extremely rare today, thus reducing the need for ICU readmission. Mortality of HT patients who are readmitted because of infection is high (56%).

P1142 Isolation of Asaia sp. in two bacteraemia cases in patients with idiopathic dilated cardiomyopathy awaiting heart transplants

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Objectives: The genus Asaia consists of 3 species, A. bogorensis, A. krungthepensis, and A. siamensis, all described as acetic acid bacteria and originally isolated from tropical flowers in SE Asia. A. bogorensis is the only species that has been associated with human disease, in a case of bacteraemia and another of peritonitis. A fourth species designation, "A. lannaensis", is unpublished; however, a corresponding sequence has been deposited in GenBank. Here we describe the first two cases of nosocomial infections with Asaia sp., closely affiliated to "A. lannaensis".

Case study: Two patients, 15 months and 5 years old, with idiopathic dilated cardiomyopothy awaiting cardiac transplantations in one unit at Arkansas Children's Hospital developed bacteraemia. Both patients within one week developed similar symptoms, such as high grade fever, hypotension, leukopenia, and thrombocytopenia. Multiple blood cultures drawn from PICC lines, central venous lines and a peripheral culture (from one patient) were positive for Gram-negative rods. Isolates from both patients were non-motile, non-hemolytic, strictly aerobic, catalase positive and showed negative reactions for oxidase, indole, urea and esculin hydrolysis. The pink pigmented colonies grew on blood and chocolate agar, but not on MacConkey and Mueller-Hinton. Growth was seen at 25 and 35ºC, but not at 42ºC. The BD Phoenix identified the organisms as *Pseudomonas luteola* and *Acinetobacter baumannii* with confidence values of 99 and 90%, respectively. The Remel rapID NH kit resulted the pathogen as *Gardnerella vaginalis* with a probability of >99.9% in each case. Non-matching biochemical results excluded these organisms for true identification. The organisms were submitted to the CDC for 16S rDNA sequence analysis and extensive biochemical tests revealing that both strains are 100% identical to each other. Further phylogenetic analyses showed the two unknown organisms to be 100% identical with A. lannaensis and a 99.6% similarity with A. bogorensis. Both patients received 14 days of meropenem and had their lines removed. Subsequent both patients recovered and received heart transplants.

Conclusion: This is the first report of human infection with the bacterium "A. lannaensis". The physical location of both patients, the temporal association of infections as well as the isolation of two identical pathogens suggests a nosocomial infection. An epidemiological investigation did not identify a source of this organism.

P1143 *Aspergillus* galactomannan antigen in liver transplant recipients: a high false-positive rate in serum samples obtained during the first week post-transplantation

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Background: *Aspergillus* galactomannan antigen (GM) is a useful tool as early marker of patients at risk to develop invasive aspergillosis (IA),

but it could have false-positive (FP) results. The causes of FP reported are varied like some foods, altered intestinal barrier, other types of fungi and semi-synthetic β-lactam treatments, especially piperacillin-tazobactam.

Methods: From jul-03 to Apr-07, a prospective study, testing periodically GM in serum samples of liver transplant recipients (LTR), was performed in our Institution. Platelia *Aspergillus* EIA-sandwich microplate assay was used following the manufacturer recommendations. Samples with an index >0.5 were considered positive. GM test was performed weekly during the 1st month after transplantation. Uni- and multivariate analysis were performed to evaluate the relation of false positive GM with clinical circumstances or therapeutic procedures after transplantation. In vitro studies including diferent β-lactams were tested for the presence of Asp-GM by use of Platelia *Aspergillus* test.

Results: During this period, 3 pts (3.4%) had a diagnosis of IA and GM were positive in 2 of them. After excluding patients with IA, a total of 414 serum samples from 85 LTR were analysed. Mean number of samples per pt was 4.8 (median:4.0). The number of FP of GM was 40 (9.6%), corresponding to 28 pts. FP GM in samples obtained during the 1st week post-transplantation was 36% (27 of 75), in comparison to 3.8% (13 of 339) in samples obtained after the 1st week ($p < 0.001$). Multivariate analysis showed that prophylaxis with ampicillin was the only independent factor related with a FP GM. In vitro studies showed a positive GM results (>0.5) in 4/6 ampicillin vials, in 3/6 piperacillin-tazobactam vials, in 0/6 cefotaxime vials and in 0/6 controls.

Conclusions: The frequency of FP GM in LTR in our centre was significantly high during the 1st week post-transplant. The present study suggests that ampicillin administered as prophylaxis to our patients during the first 2 days could be the cause of FP GM. Due to the timing of IA in these patients, it seems advisable and safety to delay GM monitorisation in these patients

P1144 Prospective study of infectious complications in a cohort of 54 consecutive allogeneic haematopoietic stem cell transplant recipients

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Objectives: To determine the incidence, aetiology and mortality during the first 2 years after transplantation in allogeneic stem cell transplant recipients (allo-HSCT).

Methods: Prospective, observational study of a consecutive allo-HSCT adult recipients cohort, between July-03 and May-06. Descriptive analyses of infection and mortality.

Results: 54 consecutive allo-HSCT recipients were included, median age 40.5 years (16–58), 52% males. Median follow-up was 180 days (9–720). Eighty-two infections occurred in 41 patients. Incidence of infection was 1.4 episodes/patient during the first year and 2 episodes/patient during the first 2 years after transplantation. In the pre-engraftment period (PEP) the incidence of infection was 0.5 episodes/patient, in the mid recovery period (MRP) was 0.6 and in the late recovery period (LRP) 0.8. In the PEP there were 29 infections (35.4%), and the most frequent were catheter-related (34.5%), pneumonia (17.2%) and bacteraemia (17.2%). In the MRP there were 27 episodes (32.9%), the most frequent were CMV infection (42.3%), and primary bacteraemia (22.2%). In the LRP there were 26 episodes (31.7%), being the most frequent CMV infection (23.1%) and mucocutaneous infection (23.1%). Etiological diagnosis was established in 65/82 episodes (79.3%). Bacterial aetiology predominated (55.4%), followed by viral (40%) and fungal (4.6%). In the PEP Gram-positive bacteria were the most frequent aetiology (42.8%), followed by Gram-negative (33.3%). There were 2 invasive aspergillosis (9.5%). In the MRP Gram-negative bacteria predominated, together with viruses, all of them CMV (42.3%). In the LRP the most frequent aetiology were viruses (66.6%) mainly CMV (33.3%) followed by VHS (22.2.%). Thirteen patients died during the follow-up (24%) The mortality rate by periods was: 5.5% (PEP), 11.8% (MRP) and 8.9% (LRP). Death was infection-related in six cases (46%).

Conclusions: The incidence of infection after allo-HSCT is high and reaches 2 episodes per patient up to 2 years after transplantation. The most frequent infections in the pre-engraftment period are catheter-related and pneumonia, whereas in mid and late recovery periods is CMV infection. The most frequent aetiology are bacteria, but viruses increase in frequency along the recovery periods and are the most frequent in the late recovery period. Mortality is high, and predominantly related to infection.

P1145 Comparison of quantitative PCR after automatic DNA extraction and pp65 antigen detection for diagnosis of CMV infection in stem cell transplant recipients

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Objectives: Cytomegalovirus (CMV) infection has been recognised as one of the major causes of morbidity in stem cell and solid organ transplant recipients. Efficacy of treatment is closely related to accurate early diagnostic laboratory tests. Quantitation of CMV DNA by conventional polymerase chain reaction (PCR) and pp65 antigen detection in peripheral blood specimens are performed in our department. The aim of this study is the comparison and correlation between the two methods in the diagnosis of CMV infection in our stem cell transplant recipients.

Methods: We retrospectively reviewed peripheral blood samples from stem cell transplant recipients obtained between January 1st 2005 and September 10th 2007. Quantitative PCR was performed using COBAS Ampliprep TNAI kit® (Roche) after DNA automatic extraction, and pp65 antigen was detected by CINApool Anti HCMH ppUL83® (Argene) kit. A positive sample was defined by a pp65 antigenaemia $\geqslant 2$ cel/4×10^5 cel and/or PCR $\geqslant 600$ copies/mL. Episodes were defined as serial positive samples until continued negativity for at least 15 days. Samples analysis and episodes analysis were performed independently. Qualitative variables are expressed as proportions. Concordance between both methods was determined by testing Kappa index and Spearman coefficient. P-values lower than 0.05 were considered statistically significant.

Results: We studied 439 samples, identifying 84 episodes from 53 different patients. Samples analysis: 10.3% of pp65 determinations were non-evaluable and 1.1% of PCR were invalidated. 212 (48.3%) positive samples were detected (18.4% were detected only by pp65, 33.5% only by PCR and 48.1% by both methods). Correlation between pp65 and PCR was medium (Spearman coefficient=0.37, $p < 0.001$). Assuming qualitative interpretation of pp65 and PCR, medium concordance was also found (Kappa index=0.42, $p < 0.001$). Episodes analysis: 19.0% were detected only by pp65, 16.7% only by PCR and 64.3% by both methods. 36.9% episodes were detected at the same time by PCR and pp65. 8.3% were early detected by pp65 and 19.0% by PCR, and in both cases with a median of 7 days.

Conclusions: Medium correlation has been found between pp65 antigen detection and conventional PCR. An important proportion of samples and episodes have been detected only by one test, so both methods are complementary, and PCR cannot replace antigenaemia.

P1146 Evaluation of the cytomegalovirus reactivation after solid organ transplant

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Objectives: The goal of this work was to determine in solid transplant recipient patients whether or not the valganciclovir preemptive therapy can protect from CMV late disease allowing the early activation of the specific immune response against CMV in T cells.

Methods: We studied solid transplant recipients with high risk for CMV infection. Patients with negative serotype for CMV receiving a CMV-positive transplant were followed. From week two post-transplant, CMV viral load (VL) was determined periodically in blood, using real time PCR with CMV especific fluorescent-primers compared to known

standards. The results of the PCR were used to guide the administration of preemptive treatment with valganciclovir. The same samples were used to quantify CMV VL by the antigenaemia test. We also measured the T cells especific immune response against CMV by flow citometry, detecting especific cell surface markers (CD69 and CD3) and citokines production (IL-4 and IFN-gamma).
Results: We followed a total of 113 transplants (kidney: 55, liver:43 and heart:15). Two of the liver transplant patients had the features for the study. In the first patient, VL was detected by real time PCR 20 out of the 22 weeks (non-consecutives) analysed (VL from 3 to 97975 copies/ml), begining two weeks after the transplant. However, the antigenaemia test was positive on week 5 post-transplant and only two more consecutive weeks. Guided by the real time PCR results valganciclovir preemptive therapy was administered in two cycles from week 3 to 9 and from week 11 to 15 respectively. The CMV especific immune response was positive at week 22 after the transplant, which could contribute to the cleareance of a CMV rebound between weeks 22 and 25 without the administration of valganciclovir.
In the second patient, the PCR was positive 10 out of the 13 (non-consecutives) weeks analysed (VL from 4 to 28907 copies/ml) begining four weeks after the transplant. Again, the antigenaemia test was positive only on week 5 post-transplant. Valganciclovir preemptive therapy was administered from week 4 to 11 and no especific immune response has been detected yet.
Conclusion: Real time PCR provides a rapid, accurate and more sensitive method for early detection of CMV VL, allowing better monitoring of solid transplant recipient patients and early administration of valganciclovir preemptive therapy. Furthermore, in one of the patients the we detected early activation of the especific immune response against CMV.

P1147 Phenotypic and genotypic characterisation of *Neisseria gonorrhoeae* isolated from HIV positive and negative patients in Italy: emergence of ST 661 clone

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Objectives: Reliable data concerning the incidence of *Neisseria gonorrhoeae* infections in Italy is lacking, as is information about the phenotypic and genotypic characteristics of circulating strains. Gonococcal isolates (n= 401) collected from patients with or without concurrent HIV infection, were characterised by serovar determination and antimicrobial susceptibility testing. Selected isolates were studied by opa typing, porB sequence analysis and *Neisseria gonorrhoeae* Multi Antigen Sequence Typing (NG-MAST).
Methods: The methods used for phenotypic and genotypic characterisation of the strains were already described in the literature.
Results: The isolates were assigned to three different serovars (IA, IB, IA/IB), with IB the most frequently encountered. Ciprofloxacin, penicillin and tetracycline resistance phenotypes were detected as well as resistance to two or more drugs. Two main opa types were identified accounting for 79.7% among the analysed strains.
Despite the presence of different STs (as identified by NG-MAST), some never detected in other countries, genetic characterisation of the circulating strains showed the persistence of ST661 in a specific geographic area. This large cluster might indicate that re-assortment and DNA recombination are not extremely frequent among strains circulating in that geographical area or alternatively, isolates of this genotype have a fitness advantage over other strains circulating enabling it to persist in the population. The association of this cluster to a drug resistant phenotype could have negatively influenced the infection of individuals, a particular concern in the case of HIV positive patients. The chain of transmission was well established for isolates identified as ST661, as evidence of the power of NG-MAST in the genetic study of gonococci. These results support previous reports on the rates of individuals co-infected with HIV and the circulation of resistant gonococci.
Conclusion: In conclusion, no correlation was found between specific gonococcal phenotypic or genotypic traits with co-infection with HIV-1. Moreover, a large cluster of resistant isolates was detected in Italy despite a low incidence of gonorrhoea. The combination of microbiological and epidemiological data enables the identification of clinically significant strains circulating that would otherwise not be disclosed, such as the ST661 cluster.

P1148 Predictive factors for typhlitis in paediatric oncology patients with neutropenia and abdominal focus

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Several risk factors related to typhlitis in neutropenic patients have been reported. However, few data are available in Latinamerican children. To identify independent risk factors for typhlitis we performed a retrospective cohort study in a Tertiary Care Center between 01/2003–11/2007.
Methods: Inclusion criteria: POP with neutropenia and abdominal focus (AF). Case definition: Typhlitis: AF and bowel wall thinckess ±4 mm by CT scan and/or ultrasonography. Control group: Patients that didn't fullfilled case definition. We excluded patients without AF.
Outcome: Typhlitis. Predictive factors: age, underlying disease, Cytosine arabinoside (ARA C) therapy, fever, diarrhoea, vomiting, abdominal pain and tenderness, sepsis and platelets. Hospitalisation days, microbiologic data and parenteral nutrition were also analysed. Statistical analysis: To compare categorical and continuous data between groups we used Chi-square and Mann-Whithney tests, respectively (error 0.05). The multiple regression analysis included all factors with $p < 0.05$ in the univariate model.
Results: From 82 AF episodes in 63 patients; 24 (29%) were typhlitis. Age: 126 mo (median) r5–211, male 41/82 (50%). Acute Myeloid Leukaemia (AML) typhlitis 10/24 (42%) controls: 9/58 (15.5%) p 0.01 OR 3.8 (CI95% 1.1–13). Cases differed from controls in the presence of fever 24/24 (100%) vs 49/58 (84%) p 0.04; diarrhoea 24/24 (100%) vs 44/58 (75.8%) p 0.008; abdominal pain 22/24 (91.7%) vs 38/58 (65.5%) p 0.01; abdominal tenderness 17/24 (70.8%) vs 10/58 (17.2%) p 0.001; sepsis 14/24 (58.3%) vs 18/58 (31%) p 0.02; platelets 18,000/ul (median) r3,000–370,000/ul vs 48,000/ul r6,000–244,000/ul $p < 0.01$ and ARA C 17/24 (70.8%) vs 24/54 (44.4%). No statistical difference in vomiting and epigastric pain. In the multivariate analysis, factors that remained significantly associated with typhlitis were abdominal tenderness p 0.02 OR 3.1 (CI95% 1.1–8.6) and abdominal pain p 0.02 OR 12.8 (CI95% 1.5–109). Microbiologic agents in typhlitis 9/24 (37.5%) vs 9/58 (15.5%) p 0.03. *Klebsiella* sp. and *E. coli* were most prevalent. Duration of hospitalisation in typhlitis:16.3 d (median) r2–60 vs 10 (3–60) p 0.001. Parental Nutrition in typhlitis 17/24 (70.8%) vs control 3/58 (5.1%) $p < 0.001$. Mortality: 3/82 (3.6%), tiphlitis 2/82.
Conclusions: In POP with neutropenia, images to confirm typhlitis should be done when abdominal pain and tenderness are present. Typhlitis was more frequent in patients with AML, ARA C and severe trombopenia.

P1149 Incidence and risk factors of infection in a single cohort of 110 adults with systemic lupus erythematosus

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Objectives: Systemic lupus erythematosus (SLE) is a chronic, usually life-long, potentially fatal autoimmune disease characterised by unpredictable exacerbations and remissions with variable clinical manifestations. Although there is a high probability for clinical involvement of other various organs in patients with SLE, infectious diseases have emerged as one of the leading causes of morbidity and mortality, accounting for 14–45% for SLE-related deaths.
A number of factors have been reported to increase risk of infection in SLE patients. While previously studies have focused on describing risk factors, data regarding predictors of infection at the time of SLE

diagnosis or immediately before the infectious event is deficient. The aims of this study are to describe infectious complications and analyse their risk factors at the time of SLE diagnosis using a single cohort of 110 adults with SLE.

Methods: A retrospective review of 110 patients who met the American Rheumatism Association criteria for SLE from June 1991 to January 2000 was performed. The records were analysed to determine the risk factors for infection by comparing patients who had suffered at least one infectious episode (n = 32; 38%) with patients that had none(n = 38; 60%).

Results: The incidence of infection in 110 patients with SLE was 4.4/100 patient-year. Pneumonia constituted 33 percent (14 cases) of all infectious complications and the most frequently yielded pathogen was *Streptococcus pneumoniae*(20%, n = 3). According to univariate analysis, significant risk factors for infection were: high SLEDAI score, total accumulated dose of corticosteroids, follow-up duration, initial C3/C4/anti-dsDNA antibody/WBC count, frequent flares. Multivariate analyses retained SLEDAI score (⩾13, p = 0.01), C3 (<90 mg/dl, p = 0.01), positive anti-dsDNA antibodies(p < 0.01) at the time of SLE diagnosis as independent predictors for infection and frequent flares(p = 0.04), follow-up duration (⩾8 years, p = 0.023) as independent risk factors for infection.

Conclusion: In lupus patients, infections are most often caused by community acquired bacteria. SLEDAI score, C3 level, anti-dsDNA antibodies are independent predictive factors and disease flare rate, follow-up duration are independent risk factors of infection. On the basis of our findings, it seems mandatory to closely observe SLE patients with factors mentioned above.

P1150 Trends in bacterial isolates from blood cultures and their resistance in relation to the antibiotic consumption at the haemato-oncological department in the period 2000–2006

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Objectives: The indiscriminate use of antibiotic (ATB) has contributed to increase the antimicrobial resistance, which has significant influence on mortality rate in immunocompromised pts. Continuous monitoring of pathogens, resistance and consumption of ATB is of cardinal importance for empirical ATB use in hematooncological pts.

Methods: The aim of the study was the retrospective analysis of trends in bacterial strains and their resistance isolated from blood cultures (BC) in large hematooncological dpt. in Czech Republic in relation to the ATB consumption in 2000–2006.

Results: During the entire period the average total No. of BC per year obtained at our dpt. was 4170. The percentage ratio of positive BC from total No. of obtained BC remained around 15% all the time. Gram-positive (G+) strains predominate (70%), but 67% of them were caused by coagulase-negative staphylococci, that are in majority of samples assigned as contaminant. The Gram-negative (G–) strains caused in average 30% of bacteraemias per year during the whole period. 70–80% of all G– isolates consisted of the following strains: *Pseudomonas* sp., *Klebsiella* sp., *Escherichia* sp. and strains of G– non-fermentative bacteria. The development of ATB resistance among isolates of G– strains was following: the resistance of *Pseudomonas* sp. to carbapenems was the biggest issue in 2001 (53%), it resulted in marked decrease of their usage and enhanced usage of the third generation (III. g.) cephalosporins. This led to lower incidence of *Pseudomonas* sp. isolates from BC (2001 – 20% vs. 2003 – 13%) and to its greater sensitivity to carbapenems (2001 – 47% vs. 2003 – 73%). On the other hand No. of ESBL *Klebsiella* sp. isolates rose in 2004. Since 2000 the consumption of amikacin has been significantly increasing which resulted in much higher resistance of *Klebsiella* sp. to aminoglycosides (2000 – 16% vs. 2005 – 51%). More frequent usage of vancomycin and III. g. cephalosporins between 2000 and 2004 was in 2004 reflected in an extreme increase of vancomycin-resistant enterococci (VRE) isolated from BC (40%). An appropriate regimen and marked decrease in usage of the above mentioned group of ATB led to the elimination of VRE in the following years.

Conclusion: Continuous monitoring of isolates from BC and their resistance in relation to the usage of ATB facilitates the choice of appropriate drug for the empirical treatment of febrile neutropenia and thus results in lower morbidity/mortality of pts. and costs of ATB therapy.

	2000	2001	2002	2003	2004	2005	2006
Total No. of BC	4115	3950	3795	4786	4491	4330	3730
Positive BC (%)	18	15	14	14	13	10	14
Strains distribution							
All G+ isolates (%)	72	72	68	63	66	74	65
Staphylococcus coagulase-negative from all G+ (%)	70	71	68	67	68	65	60
All G– isolates (%)	24	25	30	34	32	24	34
Pseudomonas sp. from all G– (%)	19	20	25	13	18	14	16
Klebsiella sp. from all G– (%)	20	26	19	19	23	24	16
Escherichia sp. from all G– (%)	19	24	27	26	20	30	33
G– non-fermentative b. from all G– (%)	10	7	8	10	3	11	14
Resistance							
of *Pseudomonas* sp. to carbapenems (%)	33	53	39	27	23	20	47
of *Klebsiella* sp. to amikacin (%)	16	30	35	36	50	51	12
of *Enterococcus* sp. to vancomycin (%)	0	4	0	8	40	3	0

P1151 The values of leukocyte count, C-reactive protein, and erythrocyte sedimentation rate for diagnosis of osteomyelitis in patients with diabetic foot ulcers

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Objectives: Diabetic foot infections are serious problems in diabetic patients and osteomyelitis is not a rare complication of these infections. In this study, we aimed to investigate the diagnostic values of WBC count, C-reactive protein (CRP) level and erythrocyte sedimentation rate (ESR) for diagnosis of osteomyelitis.

Methods: Patients admitted to our hospital with diabetic foot ulcers in three years period, from January 2004 to December 2006, were included in the study. Demographical data, duration and classification of foot ulcer, WBC counts, ESRs, and CRP levels were recorded. Reference values of tests were 0–5 mg/dL for CRP and 0–20 mm/h for ESR. Osteomyelitis was diagnosed according to the radiological findings (X-ray and MRI). Sensitivities, specificities, positive and negative predictive values (PPV and NPV) of laboratory tests were calculated.

Results: In the study period 142 patients were admitted to the hospital with complaints of diabetic foot infection. Of these patients, 100 (70.4%) were male, 42 (29.6%) were female and mean age of patients was 62.28±11.34 (35–88). Duration of ulcers were shorter than one week in 19 (13.4%), 1–4 weeks in 38 (26.8%) and longer than four weeks in 84 (59.2%) of patients. Osteomyelitis was diagnosed in 52 (36.6%) patients and 35 (67.3%) patients with ostemyelitis had ulcer for more than four weeks. For diagnosis of osteomyelitis, sensitivity, specificity, PPV and NPV of WBC, CRP, and ESR levels were summarised in the table.

Table. Sensitivity, specificity, PPV and NPV percentages of laboratory tests

Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Leucocytosis	57.6	54.4	42.2	69
ESR (higher than 20 mm/h)	94.2	8.8	37.4	72.7
ESR (higher than 75 mm/h)	40.3	75.5	48.8	68.6
CRP (higher than 5 mg/dL)	90.3	15.5	38.2	73.6
CRP (higher than 100 mg/dL)	55.7	78.8	60.4	75.5

Conclusions: ESR and CRP levels had very low specificities (8.8% for ESR and 15.5% for CRP) but their sensitivities were comparatively high (94.2% for ESR and 90.3% for CRP). When different levels of ESR (higher than 75 mm/h) and CRP (higher than 100 mg/dL) were taken in

to consideration we realised an elevation in their values. We concluded that none of these tests has high sensitivity and specificity alone. But for evaluation of a patient with diabetic foot ulcer, especially with longer duration, they should be considered together.

P1152 Treatment failure of cefepime therapy for febrile neutropenic patients with bacteraemia caused by cefepime susceptible ESBL-producing organisms

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Objective: A retrospective analysis was carried out to evaluate treatment outcome of cefepime therapy for bacteraemia caused by apparently susceptible ESBL-producing *E. coli* and *K. pneumoniae*(ESBL-EK) in neutropenic patients.
Method: From January 2005 to December 2006, we selected the febrile neutropenic patients with bacteraemia due to ESBL-EK who were treated with cefepime. Antibiotic susceptibility testing was performed on using the modified broth microdilution method. For each isolates, MICs were determined for inoculum sizes of 105 and 107 cfu/ml. To characterise ESBLs, the TEM-, SHV-, and CTX-M-related genes from clinical isolates were amplified by PCR.
Result: Fifty-eight patients with ESBL *E. coli* and thirty-one patients with ESBL *K. pneumoniae* bacteraemia were identified during the study period. Of these patients, there were fifteen febrile neutropenic patients using cefepime. As a result, six eligible patients were included.

Table 1. Clinical outcomes of 6 febrile neutropenic patients with ESBL-producing *K. pneumoniae* and *E. coli* bacteraemia treated empirically with cefepime to which the causative organisms were susceptible in vitro

Age/Sex	Underlying disease	Site of infection	Organisms	Responsible ESBL	Initial antibiotic regimen	Cefepime MIC (μg/ml) 10^6 cfu/ml	10^7 cfu/ml	Treatment outcome
52/F	Multiple myeloma	Urinary tract	*E. coli*	TEM	FEP	8	>256	Cure: complete response to initial antimicrobial therapy
70/F	Lymphoma	Unknown	*K. pneumoniae*	TEM	FEP, VAN	8	>256	Failure: persistent fever and bacteraemia after 3 days; changed to imipenem, but infected by MRSA on 9th day and died on 30th day of treatment.
40/M	Aplastic anaemia	Biliary tract	*E. coli*	TEM	FEP	8	>256	Failure: persistent fever and bacteraemia after 3 days; changed to meropenem without biliary intervention and died on 20th day of treatment.
41/M	Aplastic anaemia	Lung	*K. pneumoniae*	TEM	FEP	2	128	Failure: persistent fever and bacteraemia after 3 days; changed to imipenem and transferred to a hospice hospital on 15th day.
45/F	Lymphoma	Perianal abscess	*E. coli*	TEM	FEP, VAN, MTZ	2	128	Failure: persistent fever after 3 days; changed to imipenem and vancomycin, but died on 20th day of treatment.
70/M	Lymphoma	Unknown	*K. pneumoniae*	CTX-M	FEP	2	128	Failure: persistent fever and bacteraemia after 3 days; changed to imipenem, but infected by *Candida* on 5th day and died on 24th day of treatment.

Abbreviations: FEP: cefepime; VAN: vancomycin; MTZ: metronidazole; GI: gastrointestinal.

Conclusions: The outcome of cefepime therapy for bacteraemia due to ESBL-EK was poor, even in apparently susceptible organisms. Thus, cefepime might not provide an optimal therapeutic option in ESBL-EK infections, even in apparently susceptible isolates.

Molecular diagnostics of atypical bacteria

P1153 Association of *Ureaplasma urealyticum* infection with varicocele-related infertility

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Introduction: Varicocele is a physical abnormality present in 15% of healthy men and 40% of men being treated in infertility clinics. The association between reduced male fertility and varicocele likely is related to semen abnormalities, decreased testicular volume and decline in Leydig's cell function. On the other hand, the presence of *U. urealyticum* in semen is also related to a decrease in sperm density, motility and morphology. Thus in this study, the prevalence of *U. urealyticum* colonisation in infertile patients with and without varicocele compared with healthy men and their negatives effects on semen parameters were determined.
Methods: Semen samples were obtained from infertile patients with or without varicocele and healthy control and were subjected to the routine semen analysis and PCR. DNA was extracted by Cadieux method, and analysed by PCR protocol with species-specific primers for *U. urealyticum* (urease gene).
Results: *U. urealyticum* was detected by PCR in 23 of 146 (15.75%) semen specimens from infertile patients and in 3 of 100 (3%) healthy men ($P < 0.001$). Infertile patients with varicocele had higher *U. urealyticum* colonisation [17/81(20.98%)] than those without varicocele [6/65(9.23%), P = 0.086] or healthy controls [3/100 (3%), $P < 0.001$].The percentage of sperm cells with motility, volume of semen fluid, concentration of sperm cells, and sperm cell with normal morphology were significantly decreased in infertile men ($P < 0.001$). In the group of varicocele patients with PCR positive for *U. urealyticum* the volume, count and morphology of semen samples were lower than in the varicocele patients with PCR negative results, but the differences were not significant ($P > 0.05$).
Conclusion: The results indicate that *U. urealyticum* colonisation may be additional negative factor influencing varicocele status and worsening the reproductive potential.

P1154 The prevalence of *Mycoplasma genitalium* in the Netherlands

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Introduction: *Mycoplasma genitalium* (Mg) has been recognised as the third-most important cause of urethritis, after *Chlamydia trachomatis* (Ct) and *N. gonorrhoeae* (Ng), and may also play a role in cervicitis and pelvic-inflammatory disease (PID). The goal of this study is to determine the prevalence of Mg in different populations in the Netherlands.
Methods: Study populations: (A) 163 men with non-specific complaints of urethritis (NSU) that visited the anonymous sexual transmitted infection (STI) clinic of the Health Service Amsterdam, hereafter: "high-risk population". (B) patients that visited their general practitioner (GP) in the Delft/Westland area, hereafter: "low-risk population". This population was divided in subgroups based upon information provided by their GP. (1) men with urethritis, (2) women with pelvic inflammatory disease (PID)/cervicitis, (3) men and women asking for STD-screening. Diagnostic STI-samples (urethral swabs, urine and vaginal swabs) were anonymised for this study.
Methods: Mg was detected by real-time PCR based on conserved regions of the MgPa adhesin gene as described (Jensen et al. JCM 2004 42 683–92). Positive controls were kindly provided by JS Jensen. Ct was tested by PCR, Ng was tested by PCR and/or by culture.
Results: The results are shown in the table.

Table: the prevalence of *M. genitalium* compared to *C. trachomatis* and *N. gonorrhoeae* in the different patient groups.

	M. genitalium	*C. trachomatis*	*N. gonorrhoeae*
(A) High-risk population			
♂ urethritis NSU (163)	18 (11%)	50 (30%)	1 (0.6%)
(B) Low-risk population			
♂ urethritis (240)	20 (8.3%)	46 (19%)	8 (3.3%)
♀ PID/cervicitis (304)	5 (1.6%)	10 (3.3%)	1 (0.3%)
♂ "STD screening" (315)	10 (3.2%)	26 (8.3%)	5 (1.6%)
♀ "STD screening" (297)	8 (2.7%)	14 (4.7%)	1 (0.3%)

Of the high-risk population, 18 urine samples (11%) were found positive for Mg. Of these 18, one was HIV+, one patient was also acutely

infected with HBV. Five (27%) were positive for Ct, this was similar as in the total group (30%) One Ct was an LGV-type and another was also positive for Ng. Of the low-risk population, 20 (8.3%) men with urethritis were positive by Mg PCR. A lower prevalence was found among asymptomatic men (10; 3.2%), women diagnosed with PID or cervicitis (5; 1.6%) and asyptomatic women (8, 2.7%). No double infections were found in these groups. Mg was not detected in the control samples.

Discussion: Our data confirm the previously described correlation between the presence of Mg and urethritis. The co-infections in group A underline the high-risk category these men belong to. Women with symptoms have a similarly low prevalence as women without symptoms. In each of our subgroups, the prevalence of Mg was lower than of Ct, but higher than of Ng. Routine testing for Mg is useful for urethritis in males, but less so for women in an outpatient setting.

P1155 Evaluation of four nucleic acid amplification tests for *Chlamydia trachomatis* in urine specimens

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Chlamydia trachomatis is the most common sexually transmitted infection in the United Kingdom and nucleic acid amplification testing (NAAT) is the method of choice for diagnosis. Our aim was to assess the diagnostic sensitivity and specificity of four commercially available NAATs: Roche COBAS AMPLICOR, BD ProbeTec ET, Gen-Probe Aptima 2 Combo and Artus *C. trachomatis* Plus real-time PCR. The tests were evaluated against the same specimen panel with the same reference standard using a statistically valid number of specimens to provide a confident measure of sensitivity and specificity.

The evaluation panel consisted of 2374 male and female urine specimens. Of these 1519 were negative and 595 were positive. Samples were assessed following the UK testing algorithm for *C. trachomatis* that demands retesting of all initially positive samples and defines a positive sample as one that is reproducibly positive by the test in use.

An imperfect standard was constructed using three of the four assays under evaluation (COBAS AMPLICOR, ProbeTec ET and Aptima 2 Combo). To help assess any resulting bias five different methods (denoted as options 1–5) were used to construct the standard, showing how much the sensitivity and specificity could vary. Sensitivity and specificity were assessed against the status defined by each of the options. The data below represents results from one of the five options used to define specimen status. Differences in sensitivity and specificity between each of the options were not statistically significant.

Overall, the most sensitive assay was the APTIMA 2 Combo (99.4%), followed by the COBAS AMPLICOR (96.85%), the ProbeTec ET (95.81%) and the Artus real-time PCR (94.9%). When data was analysed by sex, all assays were found to have a higher sensitivity with male urine specimens than with female urine specimens.

The ProbeTec ET assay had the greatest specificity (99.81%) followed by the COBAS AMPLICOR (98.17%), the APTIMA 2 Combo (98.15%) and the Artus real-time PCR (97.9%).

The assays evaluated achieved good sensitivity and specificity. However, we have demonstrated that there are statistically significant differences in the sensitivity of these assays. It is of note that the most sensitive test (APTIMA 2 Combo) was the least specific (99.37%) while the least sensitive (ProbeTec ET) was the most specific. This finding demonstrates the interrelationship between sensitivity and specificity inherent in all these test platforms.

P1156 Identification and characterisation of new immunoreactive proteins of *Chlamydia trachomatis*

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Objective: The serological detection of chronic urogenital infections caused by *Chlamydia trachomatis* is complicated by the lack of known immunoreactive antigens that do not share crossreacting epitopes with other *Chlamydia* species pathogenic to humans. For that reason we aimed to identify and characterise new so far unidentified immunoreactive antigens of *Chlamydia trachomatis*.

Methods: Purified elementary bodies were analysed by 2D-PAGE followed by Western blotting with sera from patients with proven pelvic inflammatory disease caused by *Chlamydia trachomatis*. Unknown antigens being identified by that method were cloned in *Escherichia coli* and characterised with panels of sera from patients with chronic urogenital infections with *Chlamydia trachomatis* as well as from healthy blood donors with positive MIF tritres for *Chlamydia trachomatis* or *Chlamydophila pneumoniae*.

Results: Besides known immunoreactive antigens like MOMP, OMP2, and HSP60 a number of new immunoreactive antigens of *Chlamydia trachomatis* were identified by 2D-PAGE and Western blotting with the defined sera. The newly identified antigens were produced recombinantly and proved to be immunoreactive. Their reactivity to the defined serum panels was compared to known immunoreactive antigens. The data revealed a high sensitivity of the newly identified antigens. They showed lower crossreactivitiy to *Chlamydophila pneumoniae* and a much better discrimination between patients with chronic urogenital infections caused by *Chlamydia trachomatis* and seropositive but healthy blood donors.

Conclusion: The newly identified antigens need to be further characterised but might be of importance in order to improve current serological approches for the diagnostics of *Chlamydia trachomatis* infections.

P1157 A new real-time quantitative PCR to detect Coxiella burnetii DNA

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Objectives: Q fever is a worldwide zoonosis due to Coxiella burnetii. The clinical presentation may be acute (pneumonia and/or hepatitis) or chronic (most commonly endocarditis). Diagnosis mainly relies on serology. However, PCR was also shown to be useful for the diagnosis of both acute and chronic Q fever.

Methods: A quantitative PCR to be used on ABI Prism 7900 Sequence System (Applied Biosystem) was developed. DNA was extracted with Magna-PureR (Roche) and the PCR was performed using 5 ml DNA and 15 ml PCR mixture. Primers and probe were selected from highly conserved sequence of the ompA gene. Development of the quantitative PCR was done using DNA extracted from a valvular sample. The quantitative PCR was then blindly evaluated with 183 DNA samples (31 biopsies, 110 sera/EDTA blood and 42 urines) provided from the “Unité des Rickettsies” (Marseille). Among them, 41 samples taken from patients with a positive Coxiella serology were positive with the Marseille home-made nested LC-PCR.

Results: Using eubacterial 16S rRNA PCR and the new quantitative PCR, the positive control was still positive when diluted 1/1,000 and 1/100,000, respectively. With the quantitative PCR, no signal was detected in presence of bacterial DNA of other bacterial species potentially present in various non-sterile clinical samples. When tested blindly on 183 samples, the specificity of the quantitative PCR was 100% (142/142) and the sensitivity was 71% (29/41). The sensitivity was 88% (7/8) on biopsies samples, 69% (20/29) on sera/EDTA blood, and only 50% on urines (2/4).

Conclusion: This quantitative PCR is a specific method that exhibits an analytical sensitivity about one hundred fold higher than eubacterial PCR. When tested blindly, the quantitative PCR exhibited a specificity of 100%, a good sensitivity on valvular samples taken from patients with Q fever endocarditis or vascular infection, and a fair sensitivity on serum samples from patients with chronic Q fever.

P1158 Antimicrobial resistance of *Mycoplasma hominis* and *Ureaplasma urealyticum* among women with vaginal discharge

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Objectives: The aim of study was to isolate *Mycoplasma hominis* (Mh) and *Ureaplasma urealyticum* (Uu) and determine the antimicrobial resistance in cervicovaginal samples of women who had complaints of vaginal discharge using Mycoplasma IST2 (Biomerieux).
Methods: There were 151 patients with vaginal discharge included in the study: 59 pregnant and 92 non-pregnant. Vaginal samples were taken from endocervical region after ecocervical mucus had been swabbed clean. Mycoplasma IST2 used for investigation of Mh and Uu provided information about the presence or absence of Mh and Uu and also their antimicrobial susceptibility to doxycycline, josamycin, ofloxacin, erythromycin, tetracycline, ciprofloxacin, azithromycin, clarythromycin and pristinamycin.
Results: Uu has been isolated in 9 (15.2%) pregnant women, while Mh has been isolated in 1 (1.7%) of them. Uu and Mh have been both isolated in 49 (83.1%) of these patients. While of 92 non-pregnantwomen, respectively 10%, 6% and 84% have been isolated.
The Uu and Mh has been shown the lowest sensitivity to ciprofloxacin with 6 (22.2%) of isolates being resistant. The mixt Mh/Uu isolates have shown high sensitivity on Erythromycin and Clarythromycin with 83% and 81.5% respectively being sensaitive.
Conclusions: A higher prevalence of Uu/Mh is noted in the vaginal swabs as well as higher resistance in the quinolones. Low level resistance to microlides and pristinamycin must be taken into account in the empirical teatment strategy.

Antibiotic susceptibility studies in Gram-negatives

P1159 Increased antimicrobial susceptibility profiles among polymyxin-B-resistant *Acinetobacter baumannii* clinical isolates

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Objective: Infections caused by multidrug-resistant (MDR) *A. baumannii* have become a major treatment challenge, requiring the re-introduction of polymyxins into clinical practice. Given this situation, the emergence of polymyxin B (PB) resistance (R) is expected. Recently, increased antimicrobial susceptibility (S) among in vitro passaging of colistin-R *A. baumannii* isolates was observed when compared with the parent colistin-S strains. The aim of this study was to compare the S profile between PB-R and PB-S *A. baumannii* patient isolates submitted to the SENTRY Antimicrobial Surveillance Program.
Methods: A collection of 5,561 *A. baumannii* clinical isolates was S tested by the CLSI broth microdilution method and the S rates of 24 epidemiologically unrelated PB-R isolates were compared to those of PB-S isolates. S rates were analysed by chi^2 test using the Epi Info™ Version 3.4.1 software package. P values <0.05 were considered to be statistically significant.
Results: The majority of the antimicrobials tested showed limited spectrums of activity (S rates, ⩽55.4%) against the PB-S *A. baumannii* group, except for imipenem (73.2%), meropenem (76.5%) and some tetracyclines. Doxycycline, minocycline and tigecycline showed the highest S rates 74.7, 91.7 and 97.0%, respectively. Overall, the polymyxin-R group showed a higher S rate for the vast majority of antimicrobials tested. This shift was not observed among those antimicrobial agents with higher activity against the PB-S group (i.e. carbapenems and tetracyclines). Statistically significant differences were observed among most drugs showing lower activity, including ampicillin/sulbactam (S rate 3× higher), aztreonam (4×), cefoxitin (20×), ceftriaxone (2×) and cefuroxime (8×). Tigecycline was highly active regardless the susceptibility to PB.
Conclusions: PB-R *A. baumannii* clinical isolates showed higher S rates when compared to PB-S isolates for the majority of antimicrobials tested. These findings suggest that possible lipopolysaccharide modifications among PB-R bacterial cells may increase permeability to the antimicrobial agents. These data may provide additional insights for combination therapeutic options and also for possible novel antimicrobial agents in drug development.

P1160 Evaluation of tigecycline resistance in a tertiary hospital

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Objectives: Tigecycline (TG), has a wide spectrum of antimicrobial activity, and has been recently used against multi-drug resistant organisms. The aim of this study was to evaluate resistance levels to tigecycline, since this antimicrobial agent has been administered in our Hospital, in multidrug-resistant (MDR) *Acinetobacter baumannii* and *Klebsiella pneumoniae* strains isolated from ICU and non-ICU patients.
Methods: From January to September 2007, 220 unique patient multidrug-resistant *A. baumannii* and 42 *K. pneumoniae* strains were isolated from different clinical samples, both in ICU and non-ICU patients. The isolates expressed resistance to aminoglycosides, fluoroquinolones, broad-spectrum cephalosporins and carbapenems. The strains were identified using the VITEK 2 automated system (Biomerieux, France). Susceptibility to minocycline was tested by the VITEK 2 and to TG by both Kirby-Bauer (zone breakpoints for 15 μg TG discs: *Acinetobacter* spp. and Enterobacteriaceae, S ⩾ 24 mm, R ⩽ 19 mm;) and E-test (MIC(90), 2 μg/ml). Clonal relatedness of all TG-resistant strains was determined by Rep-PCR.
Results: All MDR *A. baumannii* and *K. pneumoniae* were resistant to minocycline. Overall TG-resistance rates were 10.9% (24/220) for MDR *A. baumannii* strains and 11.9% (5/42) for *K. pneumoniae* strains isolated both from ICU and non-ICU patients. ICU resistance rates were 20.3% (14/69) for *A. baumannii* and 12.0% (3/25) for *K. pneumoniae*, while non-ICU resistance rates were 6.6% (10/151) and 11.7% (2/17) respectively. In addition all TG-resistant *K. pneumoniae* isolates expressed carbapenemase activity, with a positive imipenem/EDTA test. Rep-PCR revealed high relatedness of all *A. baumannii*, regardless the origin of isolation, but *K. pneumoniae* strains were not clonally related.
Conclusion: The TG-resistance rates in MDR *A. baumannii* are similar both in ICU and non-ICU patients, while *K. pneumoniae* shows higher resistance rates in ICU than in non-ICU patients. Molecular typing indicates dissemination of related resistant *A. baumannii* strains throughout the hospital. However, tigecycline remains a potent and effective antimicrobial agent for use in hospital-acquired infections.

P1161 Analysis of tigecycline activity against Enterobacteriaceae and *Acinetobacter* spp. based on patient location and clinical specimen source in Europe

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Objective: The propensity for Enterobacteriaceae (EN) and *Acinetobacter* spp. (AC) to develop antimicrobial resistance can vary with the patient location (PL) and the type of infection (as suggested by the specimen source [SS]) from where the isolates were obtained. Therefore, to investigate trends in tigecycline (TIG) activity against EN and AC, analysis of TIG activity was stratified according to PL and SS using data collected during 2006 to 2007.
Methods: Isolates were collected from locations broadly dispersed across Europe (EU; including 11 countries) and were tested centrally using broth microdilution (CLSI M7-A7). TIG activity was analysed according to PL (outpatient [OP], intensive-care unit [ICU], and inpatient non-ICU [IP]) and SS (blood [BL], respiratory [RP], skin and skin structure [SST], and urine [UR]). EN EUCAST breakpoints (BPs) were applied to all TIG results (AC EUCAST BPs do not currently exist).
Results: See the table.
Conclusion: For EN, slight variations in %S for TIG were observed among the evaluated PL and SS; however, all populations were >95% S

to TIG. For AC, the MIC90 remained ⩽2 mg/L and the %S rates for TIG varied by 10% based on the population studied. All populations of AC were >83% S to TIG, including the ICU population which is associated with reduced susceptibility. The ability of these organisms to develop resistance requires that surveillance be on-going.

Org	Category	TIG Total n	MIC range	MIC_{90}	% Susceptible (S)
EN	OP	87	0.06–2	1	98.9
	IP	497	0.06–8	1	97.6
	ICU	289	0.06–4	1	95.5
	BL	323	0.06–8	1	97.2
	RP	275	0.06–4	1	96.4
	UR	50	0.06–2	1	98
	SST	222	0.06–2	1	97.3
AC	IP	49	0.06–4	1	93.9
	ICU	49	0.06–4	2	83.7
	BL	25	0.12–2	1	92
	RP	41	0.06–4	2	87.8
	SST	38	0.06–4	2	89.5

MIC in mg/L.

P1162 In vitro activities of several antimicrobials alone or in combination against MDR *Acinetobacter baumannii* isolated from intensive care unit

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Objective: *Acinetobacter baumannii* has become an important hospital-acquired pathogen. Carbapenems have been the agents of choice for serious *Acinetobacter* infections, but unfortunately, outbreaks of *A. baumannii* resistant to all antimicrobials except the polymyxins, have been reported. This fact creates demand on the application of some combinations of older antimicrobials on that species. The objective of this study was to assess the in vitro activity of 27 antibiotics against MDR strains of *A. baumannii* isolated from ICU, and determine whether combinations of colistin with another antibiotics act synergistically against these strains.
Methods: 32 non-repetitive isolates of *A. baumannii* were included in the study. Isolates were recovered from 21–12–2006 to 18–2–2007. Antibiotic susceptibility testing was determined by the broth dilution method according to the CLSI recomendations.
Etest with imipenem and imipenem plus EDTA were used to check metallo-β-lactamase (MBL) production. We also used cefotaxime (CT)/ cefotaxime + clavulanic acid (CTL) and cefepime (PM)/ cefepime + clavulanic acid (PML) E-test® for testing ESBL production in MDR *A. baumannii* isolates.
Four strains of *A. baumannii* were selected for the synergy study. Levels of synergistic activity for colistin combined with doxycicline, rifampicin, imipenem, meropenem and tigecycline were evaluated using the checkerboard method.
Results: All the isolates showed a high level of resistance to all β-lactam antibiotics tested. With the exception of colistin (0% of resistance) there were no antibiotics with good activity against these isolates. Of the other antibiotics tested, tigecycline shows the best activity with 1.5 mg/L of CMI90 value. MBL and ESBL were present in 32 and 0 isolates respectively. All the isolates showed the same susceptibility results, suggesting an outbreak presence.
The results of the checkerboard synergy analysis for MDR strains *A. baumannii* shown partial synergy or indifferent results.
Conclusions:
1. Susceptibility pattern revealed presence of MDR *A. baumannii* outbreak in ICU.
2. Metallo-β-lactamases were responsible of carbapenems resistance.
3. In studies of synergy, the effect of the combination treatment is unclear, although the possibility of a beneficial effect cannot be excluded
4. Tigecycline and colistin seem like the best option to treat these multirresistant bacteria.

P1163 Tigecycline activity against geographically diverse *Acinetobacter* spp. displaying multidrug-resistant phenotypes across Europe

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Objective: *Acinetobacter* (AC) is generally a nosocomial pathogen. There are several risk factors among patients that can be associated with increased infection of this organism such as extremely ill patients on a ventilator, those with a prolonged hospital stay, or those with open wounds. In 2006, tigecycline (TIG) was approved in Europe (EU) for treatment of complicated skin and skin structure infections and complicated intra-abdominal infections.
Methods: In total, 104 AC isolates were obtained from 28 hospitals in eleven countries across EU during 2006 to 2007. Isolates were tested centrally by broth microdilution (CLSI M7-A7). TIG activity was analysed according to susceptible (S), non-susceptible (NS), and multidrug phenotypes [MDR; resistance to ⩾3 agents including cefepime (FEP), ciprofloxacin (CIP), gentamicin (GEN), imipenem (IMI), minocycline (MIN), and piperacillin-tazobactam (PTZ)]. Enterobacteriaceae EUCAST breakpoints (BPs) were applied to all TIG results (BPs do not currently exist for AC). CLSI (M100-S17) BPs were used to interpret all comparators (where applicable).
Results: The most common MDR phenotype was 3-drug R with concurrent R to CIP, GEN, and PTZ (12.5% of all isolates surveyed) followed by 5-drug R with concurrent R to FEP, CIP, GEN, IMI, and PTZ (9.6% of all isolates surveyed).

Org	Total N	TIG MIC (mg/L)		%S
		Range	MIC_{90}	
All	104	0.06–4	2	89.4
CIP S	38	0.06–1	1	100
CIP NS	66	0.12–4	2	83.3
FEP S	58	0.06–2	1	96.6
FEP NS	46	0.25–4	2	80.4
IMI S	77	0.06–2	1	92.2
IMI NS	27	0.12–4	2	81.5
Non-MDR	58	0.06–1	1	100
MDR	46	0.25–4	2	76.1

Conclusions: TIG maintained potent in vitro activity against AC. The TIG MIC90 ranged from 1 to 2 mg/L, regardless of resistance or MDR phenotype. Due to the resistance associated with AC, it is imperative to monitor the susceptibility patterns of this organism against new agents such as TIG.

P1164 Evolution of the antimicrobial resistance of *A. baumannii* strains isolated at an intensive care unit

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Multiresistant *A. baumannii* including resistance to carbapenems is a common cause of severe nosocomial infections.
Objectives: to evaluate the frequency and the antimicrobial resistance evolution of *A. baumannii* isolated from ICU patients during a two years period.
Methods: From July 1st 2005 to June 30th 2007 strains of *A. baumannii* were isolated from different clinical samples coming from patients

hospitalised in the seven beds multidisciplinary ICU of our hospital. The strains were identified with the biochemical galleries (API NE) and their susceptibility to the antimicrobial agents evaluated by the disk diffusion method and E-test for imipenem, colistin and tigecycline according to CLSI recommendations.

Results: 335 strains of *A. baumannii* were isolated from 93 patients in ICU. 37 of the above group patients developed *A. baumannii* bacteraemia. All *A. baumannii* strains were sensitive to colistine with MIC90 0.25 mgr/lt and to tigecycline with MIC90 1 mgr/lit. The susceptibility rate(%) to Amikacin(A), Ciprofloxacin(C), Ceftazidime(CAZ) and Imipenem(I) decreased noticeably as follow: A: from 16 to 5, C: from 17 to 0, CAZ: from 16 to 5 and I: from 67 to 23.

Conclusion: The *A. baumannii* strains isolated in ICU patients during the period 2005–2007 presented a high level of antimicrobial resistance which was often associated to a resistance to at least 3 antimicrobial agents. In some cases the strains were totally resistant and sensitive only to colistin and tigecycline. The most notable resistance was to Imipenem which increased from 33% to 77%. Infection control measures must be constant and intense to eliminate the diffusion of these strains.

P1165 In vitro testing of carbapenem-resistant *Acinetobacter baumannii* to colistin (polymyxin E)

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Objectives: Carbapenem-resistant strains of *A. baumannii* have been reported in almost all European counties. Thus more toxic agents such as polymyxins have been used as alternative therapeutic drugs against multidrug-resistant *Acinetobacter baumannii* infections. At present there is no agreement about how to look for colistin resistance. The Societe Francaise de Microbiologie uses a cut off of 2 mg/l by broth microdilution testing, whereas the British Society for Antimicrobial Chemotherapy sets a cut off of 4 mg/l or less as sensitive, and 8 mg/l or more as resistant. There are no US standards for measuring disk diffusion sensitivity to polymyxin so far whereas CLSI (formerly NCCLS) documents do currently provide interpretative criteria for minimal inhibitory concentration by broth microdilution testing of polymyxins in *Acinetobacter* spp.

Methods: Twenty-nine non-repetitive *A. baumannii* isolates with carbapenem resistance profile were obtained from patients hospitalised at different Intensive Care Units inside Clinical Hospital Centre Split during this year and tested by broth microdilution to colistin (polymyxin E). Isolates were recovered from blood cultures, urine samples and bronchial secretions. Although disk diffusion sensitivity to polymyxin is not standardised, the diameter of inhibition zone to colistin (10 μg) was measured.

Results: Out of total number of tested strains, twenty-two isolates (22/29) displayed minimum inhibitory concentration of 2 mg/l and six (6/29) isolates MIC of 4 mg/l. One isolate displayed MIC of 128 mg/l to polymyxin.

All isolates of *A. baumannii* displayed intermediate or resistant profile to imipenem (MICs 8–16 mg/L) and meropenem (MICs 8–64 mg/L). The diameter of inhibition zone to colistin by disk diffusion test was larger than 11 mm for all strains.

Conclusion: According to currently available criteria for interpretative reading for broth microdilution testing of polymixins in *Acinetobacter* spp., we found variability in susceptibility of our isolates to polymyxin. Against CLSI standard, most of our isolates (22/29) displayed susceptibility to polymyxin showing MIC values of 2 mg/l or less as sensitive. Regarding the results obtained with a disk diffusion test, we would recommend the confirmation by a dilution method, especially in the case of serious systemic infections.

P1166 High rate of non-susceptibility to tigecycline in pan-resistant *Acinetobacter* spp. from Portugal

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Objectives: Tigecycline has been described as an alternative therapeutic agent against *Acinetobacter* spp. infections, within a global increase of multidrug-resistance context. In our country multidrug-resistant *Acinetobacter* spp. isolates have been increasingly recovered, complicating the patient's clinical outcomes. In this study we evaluated the in vitro activity of tigecycline against pan-resistant *Acinetobacter* spp. (including resistance to imipenem) isolated in Portuguese hospitals.

Methods: 250 imipenem-resistant *Acinetobacter* spp. isolates were collected between 2001 and 2007, from a variety of clinical specimens of different patients attending distinct wards in five hospitals. Isolates were identified by API32GN and by sequencing the 16S rRNA gene. MICs of tigecycline were determined by agar dilution method, according to CLSI guidelines, and a susceptible breakpoint of $\leqslant$2 mg/L. PFGE (ApaI restriction enzyme) was performed. Oxacilinase genes (OXA-23, 40, 51, and 58) were sought by a multiplex PCR.

Results: From the 250 isolates studied, 248 were identified as *A. baumannii* and 2 as *A. haemolyticus*. Resistance to several β-lactams (including imipenem) and variable susceptibility to aztreonam, ceftazidime, meropenem, amikacin and tobramycin was a common feature of these isolates. MICs for tigecycline ranged from 0.25–32 mg/L, and MIC50 and MIC90 values were 4 and 8 mg/L, respectively. Only 55 (including the two A. haemolitycus) of 250 *Acinetobacter* spp. isolates (22%) were susceptible to tigecycline. Tigecycline resistance (MIC $\geqslant$8 mg/L) was observed in 67 isolates (26.8%), belonging to the PFGE patterns disseminated within different hospitals. Among these isolates, 4 presented a MIC of 16 mg/L and 7 a MIC of 32 mg/L. Noteworthy, 128 isolates were classified as intermediate to tigecycline (MIC of 4 mg/L). All OXA-23 producing isolates presented MICs to tigecycline $\geqslant$8 mg/L.

Conclusion: High rate of non-susceptibility (78%) to tigecycline, the newest antimicrobial agent for *Acinetobacter* spp. was observed, further compromising the treatment of infections by an aetiological agent currently considered the most troublesome nosocomial pathogen.

P1167 In vitro bactericidal effect of imipenem, meropenem and piperacillin/tazobactam against β-lactam-resistant *Pseudomonas aeruginosa* strains and ESBL-producing Enterobacteriaceae

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Objectives: In France, the spread of acquired multiresistance in *P. aeruginosa* (Pa) and Enterobacteriaceae (Eb) has become a great concern. Infections caused by these bacteria require early treatment with bactericidal antibiotics. Carbapenems are often the last resort alternative. The goal of this study was to compare the in vitro kill-rates of imipenem (IPM), meropenem (MPM) and piperacillin/tazobactam (PTZ) against 7 Pa strains with different β-lactam resistance mechanisms and 4 ESBL-producing Eb.

Material and Methods: The standard time-kill method was used to investigate the bactericidal activity of IPM, MPM and PTZ. The concentrations tested were the breakpoints defined by EUCAST for Eb (2 and 8 mg/L for IPM and MPM) and for Pa (4 and 8 mg/L for IPM, 2 and 8 mg/L for MPM). For PTZ: 16/4 mg/L was used for Pa and 8/4 mg/L for Eb. The following strains were studied: (i) *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922 as reference strains, (ii) seven Pa: efflux (overexpression of MexAB/OprM pump), mutation of OprD2, carbenicillinase PSE-1, extended-spectrum β-lactamase PER-1, carbapenemases IMP-1 and VIM-2 and overexpressed constitutive cephalosporinase, (iii) four ESBL Eb: *K. pneumoniae* CTX-1 and SHV-2, *E. aerogenes* TEM-24, *E. coli* CTX-M15. Bactericidal effect was defined by a 3 Log10 CFU/mL decrease in viable counts.

Results: A bactericidal effect was obtained at 6–8 hours for IPM and MPM at 8 mg/L for all Pa strains except for those with

acquired resistance mechanisms to carbapenems (mutation of OprD2 and carbapenemases). The use of lower concentrations of IPM (4 mg/L) or MPM (2 mg/L) resulted in a decrease or abolition of bactericidal effect and a frequent regrowth after 8 h. PTZ was bactericidal only for the strain displaying efflux resistance mechanism. For ESBL-producing Eb, both carbapenems were bactericidal against all strains in 4–8 hours at 2 mg/L and 8 mg/L. MEM was more rapidly bactericidal than IPM (killing activity at 2 hours for 3 strains/4 at 8 mg/L). No regrowth was observed. PTZ was not bactericidal and only a 2 Log10 decrease of inoculum was obtained.
Conclusion: IPM and MPM achieved a rapid killing activity against the most prevalent ESBL Enterobacteriaceae in France and provided a bactericidal activity at 8 mg/L against all *P. aeruginosa* strains without OprD2 mutation or carbapenemase.

P1168 In vitro activity of doripenem relative to meropenem and imipenem against Enterobacteriaceae and *P. aeruginosa* isolates from Europe (2006–2007)

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Objective: Carbapenems are widely used to treat infections involving resistant Gram-negative pathogens due to their potent activity against extended β-lactamase producing enteric bacilli and *P. aeruginosa* (PA). Doripenem (DOR), a 1-beta-methyl carbapenem, is approved to treat complicated urinary tract infections (cUTI) and intra-abdominal infections (IAI) in the US, and is currently undergoing regulatory review for the treatment of cUTI and IAI in Europe (EU), and nosocomial pneumonia both in the US and EU. This study examined the in vitro activity of DOR relative to other currently available carbapenems against Gram-negative pathogens recently isolated from EU.
Methods: During 2006 and 2007, Enterobacteriaceae (EN) and PA isolates from patient specimens were collected from 28 laboratories in 11 EU countries. All isolates were centrally tested by broth microdilution against DOR, imipenem (IPM), and meropenem (MER) at Eurofins Medinet, Inc. (CLSI; M7–A7). Data were interpreted according to CLSI M100-S17 for IPM and MER. For DOR, there are no CLSI approved breakpoints.
Results: By MIC50/90, DOR and MER were similar in activity against the tested isolates and were superior to IPM, regardless of CAZ susceptibility. The MICs of DOR and MER were lower than IPM against the EN and PA tested.

Org	Phenotype	Total n	DOR			IPM				MER			
			MIC (mg/L)			MIC (mg/L)				MIC (mg/L)			
			Mode	MIC_{50}	MIC_{90}	Mode	MIC_{50}	MIC_{90}	% S	Mode	MIC_{50}	MIC_{90}	% S
EN	All	1,912	0.03	0.03	0.25	0.25	0.25	2	99.8	0.03	0.03	0.12	100
	CAZ S	1,656	0.03	0.03	0.12	0.25	0.25	2	99.9	0.03	0.03	0.06	100
	CAZ NS	256	0.03	0.06	0.25	0.25	0.5	2	99.2	0.03	0.06	0.25	100
PA	All	533	0.25	0.5	8	2	2	32	76.0	0.25	0.5	8	85.0
	CAZ S	421	0.25	0.25	2	2	2	8	85.3	0.25	0.5	4	92.4
	CAZ NS	112	8	4	32	32	16	32	41.1	4	4	32	57.1

CAZ: ceftazidime; S: susceptible; NS: non-susceptible

Conclusion: EN from EU were highly susceptible (>99%) to the carbapenems tested, while susceptibility of PA ranged from 76% for IPM to 85% for MER, respectively. MICs of IPM were higher against both PA and EN than MICs of either DOR or MER, which were of similar activity by MIC50/90. The increasing prevalence of carbapenemases among EN and the high-level resistance commonly encountered among PA highlights the importance of continued surveillance of carbapenem activity against these organisms.

P1169 Ertapenem, imipenem, meropenem, tigecycline and fosfomycin activity against extended-spectrum β-lactamase producing Enterobacteriaceae in Austria

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Objectives: Reported here are current Austrian susceptibility data on imipenem, meropenem, ertapenem, tigecycline and fosfomycin in ESBL-producing enterobacteriaceae.
Methods: ESBL production was detected by CLSI methods and an expanded double disk diffusion synergy test in 401 non-duplicate enterobacteriaceae (collection period June 04 to Dec 06). MICs were determined by means of Etest®. Strains not fully susceptible to one of the carbapenems were checked for the presence of metallo-β-lactamases by Etest® and typed by means of random amplification of polymorphic DNA (RAPD) and enterobacterial repetitive intergenic consensus (ERIC)-PCR.
Results: Strains were isolated from urine (44%), wounds (25%), the respiratory tract (14.5%), blood (4.5%), faeces (9%), and intravasal devices (2%). The table displays susceptibility data for the species *E. coli*, *Klebsiella* spp., and *Enterobacter* spp. No metallo-β-lactamases could be detected in strains with reduced carbapenem susceptibility. In addition, these strains were not clonally related.
Conclusions: Carbapenems were the most active antibiotics and *E. coli* was the most susceptible species tested. The lowest MICs were found for meropenem. According to CLSI and EUCAST, 3.4% and 10% strains were not fully susceptible to ertapenem, respectively. Thus, its susceptibility cannot reliably be predicted by testing imipenem or meropenem. Fosfomycin and tigecycline can be therapeutic options, but empiric susceptibility cannot be safely assumed and both agents should be tested.

	MIC_{90} (mg/L)	MIC range (mg/L)	Interpretative results in % according to CLSI/FDA (EUCAST) criteria		
			S	I	R
E. coli (n = 228)					
Ertapenem	0.38	0.003–6	98.7 (95.2)	1.3 (0)	0 (4.8)
Imipenem	0.38	0.016–1	100 (100)	0 (0)	0 (0)
Meropenem	0.064	0.006–2	100 (100)	0 (0)	0 (0)
Tigecycline	1	0.125–3	99.5 (94.3)	0.4 (4.8)	0 (0.9)
Fosfomycin	2	0.064–1024	93	3	4
Klebsiella spp. (n = 99)					
Ertapenem	0.38	0.012–32	94 (92)	1 (0)	5 (8)
Imipenem	0.25	0.064–6	99 (99)	1 (1)	0 (0)
Meropenem	0.064	0.012–3	100 (99)	0 (1)	0 (0)
Tigecycline	4	0.25–24	79 (52.5)	16 (16.2)	5 (31)
Fosfomycin	32	0.5–1024	93	1	6
Enterobacter spp. (n = 49)					
Ertapenem	1.5	0.023–32	92 (65.3)	6 (12.2)	2 (22.4)
Imipenem	0.75	0.125–2	100 (100)	0 (0)	0 (0)
Meropenem	0.125	0.016–0.38	100 (100)	0 (0)	0 (0)
Tigecycline	3	0.19–8	73 (33)	24.5 (28.6)	2 (39)
Fosfomycin	96	0.16–1024	88	8	4

P1170 Fosfomycin and tigecycline activity in extended-spectrum β-lactamase producing Enterobacteriaceae with reduced carbapenem susceptibility

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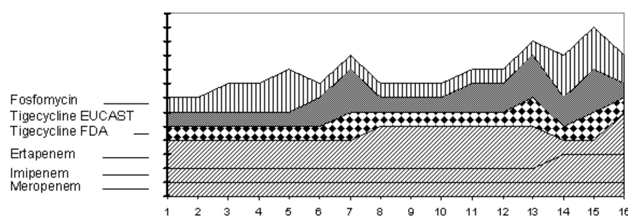
Objective: A recent Austrian susceptibility study on 401 ESBL-producing enterobacteriaceae revealed 16 (4%) and 43 (10.7%) strains (CLSI vs. EUCAST breakpoints, respectively) not fully susceptible to at least one carbapenem, the first choice agents in empiric ESBL therapy. The aim of this study was to test the in vitro activity of the new agent tigecycline and fosfomycin, which could provide therapeutic alternatives.

Methods: ESBL production was detected by CLSI methods and an expanded double disk diffusion synergy test in 401 non-duplicate enterobacteriaceae collected between June 04 and Dec 06. MICs were determined by means of Etest®. Strains not fully susceptible to one of the carbapenems were checked for the presence of metallo-β-lactamases and typed by means of random amplification of polymorphic DNA and enterobacterial repetitive intergenic consensus PCR.
Results: Meropenem was the only carbapenem not affected by reduced sensitivity. No metallo-β-lactamase production was detected in strains with reduced sensitivity to ertapenem or imipenem. According to CLSI (EUCAST) 62.5% (76.7%), 81.2% (74.4%), and 56.2% (46.5%) strains were susceptible to fosfomycin, tigecycline (FDA) and tigecycline (EUCAST), respectively. The figure displays the 16 CLSI criteria applied strains and their susceptibility to tigecycline and fosfomycin (height on y-axis stands for interpretative category: 1 space = susceptible; 2 spaces = intermediate susceptible; 3 spaces = resistant).



Strains with reduced crabpenem sensitivity according to CLSI

Conclusions: Fosfomycin and tigecycline can be therapeutic options in infections caused by ESBL-producing enterobacteriaceae with reduced sensitivity to carbapenems, but empiric use cannot be recommended and testing is mandatory. Applying EUCAST breakpoints affected significantly resistance data for tigecycline, for which every second strain out of this panel would have to be categorised as not fully susceptible. The clinical significance of this phenomenon caused by breakpoints defined by various committees remains to be clarified.

P1171 In vitro activity of temocillin vs. extended-spectrum β-lactamase-producing Enterobacteriaceae from the UK

P. Khanna, D. Wareham (London, UK)

Objectives: Temocillin is a semisynthetic pencillin derivative of ticarcillin. The methoxy group in the 6-α-position confers loss of activity against Gram-positive cocci and anaerobic Gram-negative bacilli but excellent activity against the Enterobacteriaceae family. This modification increases stability to β-lactamases, including AmpC and ESBLs. Owing to the increasing incidence of infections caused by multi-drug resistant Gram-negative bacteria and also because of the limited number of clinically active drugs available against such organisms, we aimed to determine the in vitro activity of temocillin versus clinical isolates prevalent in the UK.
Methods: Strains were identified using chromogenic media and API 20E. Susceptibility to gentamicin, ampicillin, amoxicillin/clavulanate, cefuroxime, ciprofloxacin, piperacillin/tazobactam, amikacin, trimethoprim, aztreonam, ceftazidime, nitrofurantion and imipenem was determined using the BSAC disc diffusion method. Eighty-one isolates of Enterobacteriaceae were screened for ESBL production by using double disk synergy test with cefpodoxime and cefpodoxime clavulanate. ESBLs were characterised by multiplex PCR for genes encoding CTXM3-like, CTXM-14 like and SHV-like β;-lactamases. Minimum inhibitory concentrations of Temocillin were determined by agar dilution using isosensitest agar.
Results: Eighty-one urinary isolates were tested. All isolates were resistant to cephalexin and ampicillin. 82.5% were also resistant to trimethoprim and 33.3% to nitrofurantoin, used commonly as first line agents for uncomplicated urinary tract infections. MICs to Temocillin ranged from 8–128 mg/L with an MIC50 of 8 and an MIC90 of 32 mg/L. The most common β-lactamases were CTX-M3 lilke (85.5%) followed by SHV-1 like (35.5%) and CTX-14 like (11.3%); 32.3% of isolates contained more that one ESBL. No difference in activity was seen versus strains exhibiting multi-drug resistance or the production of specific ESBLs.
Conclusions: Using the BSAC breakpoint of 32 mg/L for urinary isolates we found 93.9% of the isolates to be sensitive. Moreover, temocillin achieves urinary levels of 400–600 mg/L suggesting a considerable margin over the MICs. Thus we conclude that temocillin is a useful option for the growing number of infections, particularly of the urinary tract, that are caused by ESBL and cephalosporin resistant organisms.

P1172 Evaluation of MicroScan Neg Combo Panel Type 44 for detecting extended spectrum β-lactamases in Enterobacteriaceae

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Objectives: Accurate and rapid detection of extended spectrum β-lactamases (ESBLs) is important to guide proper patient antimicrobial therapy. The new MicroScan panel, Neg Combo Type 44 (Dade Behring, Sacramento, Calif., USA), has been developed for confirmation of ESBL-producing Enterobacteriaceae using the wells containing ceftazidime/clavulanic acid and cefotaxime/clavulanic acid. We evaluated the performance of Type 44 panel for confirmation of ESBLs.
Methods: In September 2007, 106 *Escherichia coli*, 81 *Klebsiella pneumoniae*, 11 *K. oxytoca*, and 8 *Proteus mirabilis* isolated from clinical specimens were tested with Type 44 panel. All isolates were tested with the disk approximation test (DAT) and CLSI phenotypic ESBL confirmation test (CLSI). The cefotetan-non-susceptible isolates were tested with cefotaxime and cefotaxime/clavulanate disk containing boronic acid for detection of plasmid-mediated AmpC β-lactamases (AmpC).
Result: Forty-four (21.4%) of 206 isolates were confirmed as ESBL-producers by DAT and/or CLSI including 27 *E. coli*, 14 *K. pneumoniae*, 2 *K. oxytoca*, and 1 *P. mirabilis*. Thirty-eight isolates flagged as "Confirmed ESBL" were all confirmed as ESBL-producers. Fourteen *K. pneumoniae* isolates flagged as "Possible ESBL, unable to interpret confirm test" were all confirmed as AmpC-producers. Of which, 6 (2.9%) were ESBL-producing as well as AmpC. All ESBL-producers were positively screened and ESBL-producers without AmpC were all confirmed by Type 44.
Conclusion: Type 44 warrants to be a reliable panel for detection of ESBLs in Enterobacteriaceae. The isolates that flagged as "Possible ESBL, unable to interpret confirm test" requires the confirmation for presence of AmpC.

P1173 Tigecycline activity against geographically diverse Enterobacteriaceae displaying multidrug-resistant phenotypes across Europe

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Objective: Infections derived from Enterobacteriaceae (EN) commonly occur among hospitalised patients and can be problematic to treat. Tigecycline, a new semi-synthetic derivative of minocycline and the first in the glycylcycline class of antibiotics, has previously shown activity against this family of organisms (Org). In 2006, tigecycline (TIG) was approved in Europe (EU) for treatment of complicated skin and skin structure infections and complicated intra-abdominal infections.
Methods: In total, 875 EN isolates were obtained from 28 hospitals in ten countries across EU during 2006 to 2007. Isolates were tested centrally by broth microdilution (CLSI M7-A7). TIG activity was analysed by MDR status defined as resistance to ⩾3 agents which included ampicillin, cefepime, ceftriaxone, ciprofloxacin, gentamicin, imipenem, minocycline, and piperacillin-tazobactam. EUCAST breakpoints were used to interpret TIG MIC results and CLSI (M100-S17) breakpoints were used to interpret all other agents, where applicable.
Results: See Table.

Org	category	Total N	TIG MIC (mg/L) Range	MIC_{90}	% Susceptible
EN	All	875	0.06–8	1	97.0
	Non-MDR	707	0.06–4	1	98.7
	MDR	168	0.06–8	2	89.9
E. coli	All	300	0.06–2	0.5	99.3
	Non-MDR	244	0.06–2	0.5	99.6
	MDR	56	0.06–2	0.5	98.2
K. pneumoniae	All	282	0.12–8	1	97.2
	Non-MDR	209	0.12–2	1	99.5
	MDR	73	0.12–8	1	90.4
Enterobacter spp.	All	124	0.25–2	1	91.1
	Non-MDR	96	0.25–2	1	96.9
	MDR	28	0.5–2	2	71.4
Citrobacter spp.	All	87	0.12–1	0.5	100
	Non-MDR	79	0.12–1	0.5	100
	MDR	8	0.12–1	NA	100
S. marcescens	All	82	0.25–4	1	93.9
	Non-MDR	79	0.25–4	1	94.9
	MDR	3	0.5–4	NA	66.7

Conclusions: Overall, EN was 97% susceptible to TIG. The TIG MIC90 against all EN tested did not exceed 2 mg/L, regardless of MDR phenotype. The potential for resistance to any new agent to increase with use and time dictates the need for continuous monitoring of TIG activity against this group of target pathogens.

P1174 In vitro activity of fosfomycin vs. extended-spectrum β-lactamase

A. Amin, P. Khanna, C. O'Sullivan, D. Wareham (London, UK)

Objectives: Due to the increased isolation of ciprofloxacin resistant ESBL-producing Enterobacteriaceae from the urinary tract, alternative antibiotics are urgently required. Among currently available agents, fosfomycin trometamol may be a useful agent as it concentrates in the kidney and bladder. We set out to determine the in-vitro activity of fosfomycin against both community and hospital acquired urinary tract infection (UTI) isolates from the east London Region of the UK.
Methods: All isolates collected were screened for ESBL production using double disk synergy tests with cefpodoxime and cefpodoxime/clavulanate. Strains were identified to species level using a combination of chromogenic media and API 20E identification systems. Susceptibility to gentamicin, ampicillin, amoxicillin/ clavulanate, cephalexin, cefuroxime, ciprofloxacin, piperacillin/tazobactam, amikacin, trimethoprim, aztreonam, ceftazidime, nitrofurantion, minocycline, ampicillin/sulbactam, cefoxitin, trimethorpim/sulfamethoxazole, fosfomycin and imipenem were determined using the BSAC disc diffusion method. Minimum inhibitory concentrations (MIC) of fosfomycin were determined by agar dilution using isosensitest agar. All ESBLs were characterised by multiplex PCR for genes encoding CTXM3-like, CTXM14-like and SHV-like β-lactamases.
Results: 75 urinary isolates were tested, all of which were resistant to amoxicillin and cephalexin. 81.3% were also resistant to trimethoprim, 34.7% to nitrofurantoin, and 85.3% to ciprofloxacin, 74 out of 75 isolates (98.7%) were sensitive to fosfomycin, using the BSAC MIC breakpoint of 128 mg/L. MICs to fosfomycin ranged from 0.5–>128 mg/L, with an MIC50 of 16 and an MIC90 of 32 mg/L. CTXM3-like genes were detected in 82.5% of isolates, CTXM14-like genes in 12.3%, and SHV-like genes in 36.8%.
Conclusions: 98.7% of ESBL-producing urinary isolates tested in this study were sensitive to fosfomycin, with frequent resistance to other agents including ciprofloxacin. Fosfomycin is an oral agent which is known to be concentrated in the urine. It is not nephrotoxic, is considered safe in pregnancy, and is also active against *Enterococcus* species. This study suggests that fosfomycin may be a useful option in the treatment of urinary tract infections caused by multi-resistant organisms.

P1175 Efficacy of a mixture of colloidal silver-hydrogen peroxide and 2-bromo-2-nitropropan, 1,3-diol compounds against different *Legionella pneumophila* strains

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Objectives: *Legionella pneumophila*, the causative agent of Legionnaires' disease and Pontiac fever, can be isolated from a variety of potential human contact areas (cooling tower systems, potable water sources, etc.). Because of the potential for any water system to harbour, amplify and disseminate legionellae, control measures need to be considered for all water systems. Therefore, in order to prevent legionellosis outbreaks, contaminated water systems must be disinfected and the most effective and practical biocides need to be evaluated. The aim of this study is to determine the lethal activity of various concentrations of a mixture of colloidal silver-hydrogen peroxide (CSHP) and 2-bromo-2-nitropropan, 1,3-diol (BNPD) compounds on *L. pneumophila*. The susceptibility of environmental isolates [SG 1 and SG 2–14] and standard strain to biocides was assessed at different contact times.
Methods: In the current study, the qualitative suspension test method by Skaliy et al. was modified based The American Society for Testing Materials (ASTM) E 645–91 standard test method, which is used for determining the efficacy of microbicides used in cooling systems.
Results: CSHP compound was found effective at the recommended dosages (200–500 ppm) against legionellae, since these concentrations achieved >5 log reduction at 0 hour contact time. Furthermore, all strains were killed by lower doses (100 ppm and 50 ppm) in 3 hours contact time. Recommended BNPD concentration (100 ppm) for cooling towers killed SG 2–14 and standart strains at 3 hours contact time, however SG 1 strain was killed at 6 hours contact time. The antibacterial activity of lower doses BNPD (50 and 25 ppm) against SG 1 and standard strains required longer contact times than recommended dosages.
CSHP compound was found more effective than BNPD in terms of recommended dosages and contact times.
Conclusion: CSHP compound can be recommended for decontamination of legionellae. Since, several factors such as bacterial strains, biocides and conditions in the water sytems can affect efficiency of a biocide against bacteria, activity of the biocide needs to be evaluated in laboratory conditions before practical usage.

P1176 Resistance of *Campylobacter* spp. to fluoroquinolones and macrolides in Portugal

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Objectives: When indicated, empirical treatment of diarrhoea in adults is often based on quinolones. However, the resistance of *Campylobacter* spp. to quinolones is increasing globally. Because no good data exist for Portugal, this study aimed to establish the resistance pattern in *Campylobacter* spp., isolated from human faeces.
Methods: The antimicrobial susceptibility of *Campylobacter* strains isolated from human diarrhoeal stool samples was studied. Strains isolated in Portuguese Hospital Laboratories, from 2003 onward were included. The activity of five antimicrobial drugs was tested: ciprofloxacin (CI), moxifloxacin (MX) and gatifloxacin (GA), as well as erithromycin (EM) and azitromycin (AZ). The MICs were determined with E-test strips. Since no specific breakpoints have yet been established for *Campylobacter* spp., we used the breakpoints from the Clinical Laboratory Standards Institute (CLSI) for Enterobacteriaceae (for CI and GA), for *Staphylococcus* spp. (for EM and AZ) and for *S. pneumoniae* (for MX). This allows the comparison with other studies, as these breakpoints are also used by other investigators.

Results: In this ongoing study, 123 *Campylobacter* strains were analysed so far. *Campylobacter jejuni* was the predominant species recovered: 110 isolates (89.5%), of which 66.7% were identified as *C. jejuni jejuni* 1. A total of 80.5% (99 of 123) of *Campylobacter* strains were found to be resistant to ciprofloxacin, followed by those strains resistant to moxifloxacin and gatifloxacin (51.2 and 47.2%, respectively). Interestingly, both MX and GA still showed antimicrobial activity in 29.3% and 33.3% of CI resistant cases, respectively. The MIC50s values for MX and GA were 4.0 and 6.0 μg/ml, respectively, while the value for ciprofloxacin was 32 μg/ml. A clearly different pattern was observed with respect to macrolides, to which only 6.5% of the strains studied were resistant.
Conclusion: Portugal seems to have one of the highest quinolone resistance rates in Europe, comparable with Spain (~80%). Consequently, quinolones can no longer be considered the empirical treatment of choice. If treatment is indicated, macrolides should be used, as most strains are still sensitive. However, the study raises further questions that need to be addressed: possible treatment options for the patients that show quinolone and macrolide resistance and the excessive use of quinolones in husbandry.

P1177 Comparative activity of various antimicrobial agents against *Bacteroides fragilis* group strains

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Objectives: *Bacteroides fragilis* group organisms are the most frequently isolated anaerobic bacteria in clinical infections. Increasing resistance of this group of organisms to several agents and geographical differences in resistance patterns have been reported in recent years. The purpose of this study was to asses the antibiotic susceptibility patterns of *Bacteroides fragilis* group strains isolated in our hospital.
Methods: A total of 277 non-duplicate clinical strains of the *Bacteroides fragilis* group collected from January 2005 to December 2006 were included in the study. Organisms were identified by the Rapid ID 32 A system. Susceptibilities were determined by the agar dilution method according to the CLSI criteria. The following agents were studied: metronidazole, chloramphenicol, clindamycin, tigecycline, moxifloxacin, amoxicillin-clavulanate, piperacillin-tazobactam, cefoxitin, and imipenem.
Results: Cefoxitin inhibited 63.5% of the strains at 16 mg/L. Clindamycin was the least active of the compounds tested (44.7% resistance). All isolates were susceptible to metronidazole. One isolate showed intermediate resistance to chloramphenicol, being the rest of them susceptible to this antibiotic. Tigecycline inhibited at 4 mg/L 87% of the strains. The resistance rates for amoxicillin-clavulanate and piperacillin-tazobactam were 10.1 and 2.2%, respectively. We found two *B. fragilis* isolates that were highly resistant to imipenem (MIC > 256 mg/L) and to all other β-lactam agents tested. For moxifloxacin, MIC50 and MIC90 were 1 and 8 mg/L, respectively. Among the different species of the group, *B. fragilis* was the most susceptible to clindamycin and cefoxitin, while B. thetaiotaomicron was the least susceptible to these agents.
Conclusion: The high resistance rates to clindamycin and cefoxitin and the emergence of resistance to the new β-lactams observed in this study, indicate the need of continued surveillance of susceptibility of these organisms in order to detect changes in susceptibility profiles and to select the adequate antimicrobial therapy.

P1178 Resistance of *Bacteroides* species in Trondheim, Norway

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Objectives: Bacteria belonging to the *Bacteroides fragilis* group are predominant in clinical anaerobic infections. Increasing resistance to several antibiotics has been reported in the last decades. From December 2005 until November 2007 all *Bacteroides* strains from clinical samples at our hospital were tested with Etest for five antibiotics.
Methods: The samples were cultured on fastidious anaerobic agar (FAA). Susceptibility testing was performed for Gram-negative bacteria, which were identified biochemically by rapid ID32A (BioMérieux) as *Bacteroides* species. A suspension of bacteria in Brain Heart broth of 1 McFarland turbidity was floated on PDM agar with 5% defibrinated horse blood. Etest strips (BIODISK) were applied on the dry agar surface. The MIC was read at full inhibition after 24 hours and for clindamycin after 48 hours of incubation. All culturing and susceptibility testing was performed in an anaerobic chamber and all media were prereduced.
Results: 125 isolates, cultured mainly from pus obtained by abdominal surgery and abscess drainage, were available for testing. One strain was excluded due to no growth on the Etest. The breakpoints recommended by the Norwegian Working Group on Antimicrobials (NWGA) were applied. Penicillin resistance was detected in all strains except one (*B. ureolyticus*). No strains were resistant to metronidazole, one strain (*B. capillosus*) had reduced metronidazole sensitivity. Only one strain (*B. fragilis*) was resistant to imipenem. Resistance to clindamycin and piperacillin/tazobactam was more common, 37.9% and 20.2% respectively. *B. fragilis* isolates were more sensitive to these two antibiotics than non-*fragilis* species.
Conclusion: Metronidazole and imipenem are good alternatives for empiric therapy of infections with *Bacteroides*. Species identification and susceptibility testing should be done in serious infections.

P1179 Comparison of the Etest and the Neo-Sensitabs method for antimicrobial susceptibility testing of *Helicobacter pylori*

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Objectives: To assess the reliability of the E-test (AB BIODISK) compared with the Neo-Sensitabs (Rosco) method for antimicrobial susceptibility testing of *Helicobacter pylori*.
Methods: *H. pylori* strains were susceptibility tested by both the E-test and the Neo-Sensitabs method for ampicilin, metronidazole, ciprofloxacin, tetracycline and clarithromycin.
50 clinically isolated *H. pylori* strains were grown on chocolate agar plates and incubated at 37ºC in a microaerobic gas chamber. The bacteria were harvested after 72 hours, suspended in API suspension medium (bioMérieux) and adjusted to a McFarland turbidity standard of 4 (109 CFU/ml). Inoculation was performed by applying 100 ml of the bacteria suspension to each plate (plates were room tempered before inoculation) and spread by drigalski loop. Before applying the E-test strips and the Neo-Sensitabs plates were left to dry for 15 minutes. The strips/tablets were applied using sterile forceps and only one strip/tablet per plate was applied. Incubation was conducted at 37ºC in a microaerobic gas chamber and reading after 72 hours.
In order to allow a direct comparison of E-test and tablet results, MIC (Minimal inhibitory concentration) values were first log2 transformed and then converted to scores ranging from 1 to 15 [1]. Correlations between scored MIC and zonesize were tested using Pearson's regression.

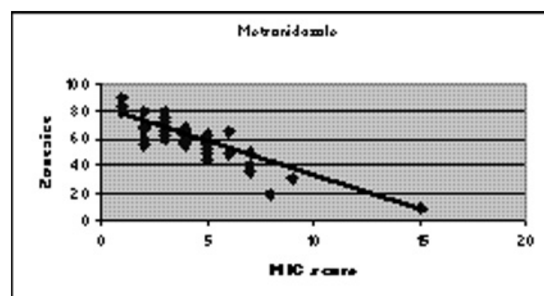

Figure 1. Correlation between metronidazole MIC values and zonesizes with 50 *H. pylori* isolates.

Results: Zonesize is plotted as a function of scored MIC of ampicilin, metronidazole, ciprofloxacin, tetracycline and clarithromycin

and correlations calculated. The corresponding correlation coefficients are –0.49, –0.97, –0.89. –0.49 and –0.67 respectively. As an example Figure 1 shows the results of metronidazole.

Conclusion: Excellent correlation was demonstrated by statistical analysis between MIC score and zonesize values for metronidazole and ciprofloxacin while the correlation was acceptable for ampicilin, tetracycline and clarithromycin.

Reference(s)

[1] Glupczynski et al, 2002. Comparison of the E Test and Agar Dilution Method for Antimicrobial Susceptibility Testing of *Helicobacter pylori*.

P1180 Clarithromycin resistance in *Helicobacter pylori* positive patients after therapies failure

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Objectives: Patients with peptic ulcers who are also infected by *Helicobacter pylori* receive antimicrobial therapy, based on different antibiotic combination including clarithromycin in addition to antisecretory drugs. Eradication is however difficult because of primary and secondary resistance. The aim of the study is to evaluate in vitro susceptibility of *H. pylori* strains isolated in a group of symptomatic patients with failure of previous eradication treatments.

Methods: 77 adult patients (27 males and 50 females) with peptic ulcers and other gastric disorders after failure of previous treatment regimens were studied. The mean age of the subjects was 54.7 years and the range 31–77 years. During endoscopy, four biopsies were taken from antrum for histology, rapid urease test and culture and two from fundus for histology and culture. A total of 144 gastric biopsies were collected. The specimens were homogenised and streaked on fresh blood agar plates and on Dent agar. The plates were incubated in a microaerophilic atmosphere at 37°C for 4 up to 10–14 days. Fresh culture of 3 days were tested for susceptibility to clarithromycin, amoxicillin, metronidazole and tetracycline by agar disk diffusion technique and by E-test method. The plates were incubated in a microaerophilic atmosphere at 37°C for 3 days.

Results: *H. pylori* was isolated from 46 (59.7%) patients; in the other subjects the culture showed no growth or contamination. Best results of isolation were obtained in blood agar plates, but Dent agar was superior when bacterial contamination was present. Clarithromycin resistance was found in 80% of the patients, metronidazole in 75% and amoxicillin in 2.5%. All strains were susceptible to tetracycline. Results obtained by agar disk diffusion technique and by E-test were concordant. Resistance to at least one drug was observed in 95% of subjects. Resistance to both clarithromycin and metronidazole was found in 57.5% of the cases. All patients with clarithromycin resistance were previously treated with clarithromycin and amoxicillin.

Conclusion: The percentage of clarithromycin resistant strains from patients with previous therapies failure is very high (80%); this percentage grow up to 100% in subjects previously treated with this drug. Resistance to tetracycline was never observed here. As this antibiotic should be used in combined alternative treatment, it should be considered in susceptibility testing.

P1181 Comparison of two disc diffusion methods with minimum inhibitory concentration for antimicrobial susceptibility testing of *Neisseria gonorrhoeae* isolates

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Background: Gonorrhoea remains a public health problem in developing countries. Antimicrobial susceptibility testing of *N. gonorrhoeae* helps in monitoring the trends and efficacy of treatment guidelines for syndromic management of gonorrhoea. Antimicrobial susceptibility testing of *N. gonorrhoeae* isolates is widely performed as per the CLSI guidelines or by the AGSP method. Results of antimicrobial susceptibility testing by disc diffusion method should be reliable when compared to MIC values.

Objective: The present study was carried out to compare two disc diffusion methods (CLSI and AGSP) with MIC determination for antimicrobial susceptibility testing of gonococcal isolates.

Methods: A total of 100 *N. gonorrhoeae* isolates were studied. The strains were isolated from 89 (86.4%) out of 103 men with urethritis, 5 (12.8%) out of 39 women with endocervicitis and 6 (46.1%) out of 13 sexual contacts who attended the STD clinic of Lok Nayak Hospital, New Delhi between January 2004 and June 2006. All isolates were examined for susceptibility to penicillin, ciprofloxacin, tetracycline, ceftriaxone and spectinomycin by the disc diffusion methods (CLSI and AGSP). MIC was determined by E-test method. β-lactamase production was assayed by chromogenic cephalosporin method. Quality control strains were included for both disc diffusion and MIC methods.

Results: All isolates were susceptible to ceftriaxone and spectinomycin while 99 (99%) were resistant to ciprofloxacin by both disc diffusion methods. Thirty-two (32%) strains were found to be resistant to penicillin by CLSI method, while 40(40%) were resistant to penicillin by AGSP method. Seventeen (17%) isolates were PPNG and 20 (20%) were TRNG. All PPNG strains had no zone of inhibition by AGSP, while they had an inhibition zone ± 19 by CLSI. There was complete agreement between results of antimicrobial susceptibility testing by both disc diffusion methods and MIC values for all antibiotics except penicillin for which the kappa was 0.7 for AGSP and 1 for CLSI.

Conclusion: The continual spread and ongoing emergence of resistance among *N. gonorrhoeae* isolates require that an accurate and simple test be performed to determine antimicrobial susceptibilities. Both disc diffusion methods showed a high degree of agreement with MIC values. Although AGSP method is more economical, its feasibility depends on the availability antimicrobial discs of low potency and it requires more accuracy in measuring the inhibition zones.

P1182 Antimicrobial susceptibility of Gram-negative bacteria causing nosocomial infections from 2003–2005 in a university hospital in Turkey

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Objective: The aim of this study was to determine about antibiotic susceptibilities of Gram-negative bacteria isolated from nosocomial infections.

Methods: The activity of eight broad-spectrum antimicrobial agents were assessed against 397 Gram-negative aerobic bacteria isolates collected from nosocomial infections during 2003, 2004 and 2005 in an university hospital in Turkey. Gram-negative bacteria were including *E. coli*(22.6%), *K. pneumoniae* (21.9%), *P. aeruginosa* (20.9%), *Acinetobacter* spp. (20.1%), *Enterobacter* spp. (7.3%), *Proteus* spp. (3.2%), *Morganella* spp. (1.2%), *Providencia* spp. (1.0%), *Serratia* spp. (0.7%) and *Citrobacter* spp. (0.5%). Antimicrobial susceptibility was investigated using E-test. Extended-spectrum β-lactamase (ESBL) production was determined using ceftazidime and ceftazidime/clavulanic acid E-test strips.

Results: Overall, meropenem and imipenem were the most effective antibiotics against Gram-negative organisms, respectively (77.5% susceptible, MIC50 = 0.125, MIC90 ⩾ 32 mg/L; 75.8% susceptible, MIC50 = 0.5, MIC90 ⩾ 32 mg/L); these were followed by piperacillin/tazobactam (56.4% susceptible; MIC50 = 16, MIC90 ⩾ 256 mg/L), ciprofloxacin (44.8% susceptible; MIC50 = 3, MIC90 ⩾ 32 mg/L), cefepime (42.8% susceptible; MIC50 = 24, MIC90 ⩾ 256 mg/L), tobramycin (39.2% susceptible; MIC50 = 16, MIC90 ⩾ 256 mg/L), ceftazidime (38.5% susceptible; MIC50 = 24, MIC90 ⩾ 256 mg/L) and cefotaxime (25.4% susceptible; MIC50 ⩾ 32, MIC90 ⩾ 32 mg/L). When these were compared with data between 2000–2002, the antibiotic susceptibilities were determined approximately lower 10% for imipenem, meropenem, piperacillin/tazobactam; 15% for cephotaxime; 20% for ceftazidime, cefepime, ciprofloxacin and tobramycin in 2003–2005. The rates of production of ESBL were 77% in *K. pneumoniae* and 61% in *E. coli*. These ESBL

positive isolates were sensitive 100%, 98% to meropenem; 98.5% and 98% to imipenem, respectively. Multi-drug resistance (MDR) rates were 76% in *Acinetobacter* spp., 66% in *P. aeruginosa*. Only piperacillin/tazobactam were more susceptible than 50% against *P. aeruginosa*. Carbapenems were the most active agents against *Acinetobacter* spp. (meropenem 57.5%, imipenem 55% susceptible) AmpC β-lactamase was produced by 31.4% of *Enterobacter* spp., *Citrobacter* spp. and *S. marcescens*. All of these were sensitive to meropenem; 77%, to imipenem and ciprofloxacin.
Conclusion: Antimicrobial resistance has reached very high levels in hospital. Solving of this problem depends primarily on to prevention of the development of antimicrobial resistance.

P1183 In vitro activity of tigecycline against multidrug-resistant Gram-negative clinical isolates

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Objectives: To evaluate the antimicrobial performance of tigecycline against multi-drug resistant (MDR) Gram-negative (GNs) clinical isolates.
Methods: A total of 78 MDR GN bacterial stains were studied. They were isolated from various clinical specimens, during a 10-month period (2/07–11/07), from both ICU and non-ICU patients. Only one isolate per patient was included in the study. *P. aeruginosa* and Proteae strains were excluded, as tigecycline exhibits reduced antimicrobial potency to these species. Species identification and MIC values determination for 37 antibiotics were performed by the VITEK 2 Compact Automated System (bioMerieux, France). The MICs for tigecycline and ampicillin/sulbactam were determined by the E-test method (AB Biodisk, Sweden). The susceptibility breakpoints for tigecycline were those approved by the FDA.
Results: The majority of the MDR strains derived from ICU-patients (51/78, 65.4%) as expected, followed by surgical patients (19/78, 24.4%). They were isolated from blood (35/78), pus (19/78), catheters (11/78), bronchial secretions (7/78) and other sources (6/78). The bacterial species were: *K. pneumoniae* (31/78), *A. baumannii* (27/78), *Enterobacter* spp. (10/78), *E. coli* (5/78) and others (5/78). Out of the 78 MDR strains five were pan-drug resistant and ten of them were susceptible only to colistin. Susceptibility to tigecycline was detected to 4/5 of pan drug resistant isolates (one was intermediately susceptible) and to 6/10 of colistin-only susceptible isolates (two of them were tigecycline resistant). MIC90 and MIC50 for tigecycline was 2 and 0.75 mg/l respectively (MIC range: 0.064–8 mg/l). *Acinetobacter baumannii* exhibited higher MIC90 and MIC50 values (3 and 2 mg/l, range: 0.125–8 mg/l) compared with *Klebsiella pneumoniae* (1, 5 and 0, 38 mg/l, range: 0.094–8). Resistance to tigecycline was detected to 2 MDR isolates (2, 6%) with MICs of 8 mg/l, while in 5 strains (6.4%) MICs were 3–4 mg/l. Among ICU-patients 8 were treated with tigecycline. No significant differences were observed to the MIC values of tigecycline for the strains derived from these patients before and after treatment.
Conclusion: The emergence of multidrug-resistance poses a serious threat. Tigecycline may represent an option for the treatment of infections caused by MDR GNs where our choices are limited. Any potential for selection of resistance should be monitored.

P1184 Current susceptibility patterns for *H. influenzae* and M. catarrahlis isolates from Europe: findings of the 2007 GLOBAL surveillance program

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Objective: *Haemophilus influenzae* (HI) and *Moraxella catarrhalis* (MC) associated with community-acquired respiratory tract infections can become resistant to commonly prescribed agents and these resistance (R) rates can vary according to regional distributions. The GLOBAL Surveillance (SUR) initiative was undertaken to track R patterns among respiratory pathogens. This study tracks the resistance of HI and MC collected as part of GLOBAL '07 to empirically used oral agents.
Methods: During 2007, 858 HI and 163 MC isolates from patient specimens were collected from 6 European (EU) countries: Belgium, France, Germany, Italy, Spain (SPN), and the United Kingdom. All isolates were centrally tested by broth microdilution according to CLSI M7-A7 and the results were interpreted according to CLSI M100-S17. Data were analysed according to β-lactamase (BL) status as positive (+) or negative (−).
Results: For all EU countries combined, 130 HI isolates were BL+ (15.2%) and 728 isolates were BL− (84.8%). Overall, current MIC90s (mg/L) were >8 for ampicillin (AMP), 0.03 for levofloxacin (LFX), and >4 for trimethoprim-sulfamethoxazole (SXT), regardless of BL phenotype, except for BL− isolates against AMP (MIC90, 0.5 mg/L). The current overall percent R among the oral agents were 15.4% AMP, 0.1% LFX, and 18.3% SXT. By country, LFX retained potent activity against HI, regardless of phenotype, based on MIC90s (0.015–0.03 mg/L for all) and susceptibility (%S) was 100% for all, except SPN (overall %S=99.4), where 1 isolate was non-susceptible. One HI isolate was found to be BL− ampicillin-resistant. For all EU countries combined, 160 MC isolates were BL+ (98.2%), with <2% BL−. With the exception of ampicillin and cefuroxime, all agents were highly active against the tested MC, with 100% susceptibility rates.
Conclusion: Among HI, R to SXT and AMP (BL mediated) was prevalent throughout EU. In contrast, current %S to LFX remains high throughout EU against HI. Against MC, isolates are 100% susceptible to all agents, with the exception of ampicillin and cefuroxime, regardless of BL+ rates among MC which have reached 98%. Continued SUR is warranted to monitor any changes that may occur among these respiratory pathogens to help guide empiric therapy for community-acquired respiratory tract infections.

P1185 Comparison between AST-N058 card (VITEK 2) and UNMIC/ID-62 panels (BD Phoenix) ESBL test for detection of extended-spectrum β-lactamases in *E. coli* and *K. pneumoniae*

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Objective: The aim of this study was to evaluate the abilities of the VITEK 2 and BD Phoenix in detecting ESBL production.
Methods: One hundred and ninety eight clinical isolates of ESBL producers (178 *E. coli* and 20 *K. pneumoniae*) were assayed by VITEK 2 (BioMérieux, France) and BD Phoenix System (Becton-Dickinson Biosciences, Sparks, Md) using AST-N058 cards and UNMIC/ID-62 panels, respectively. Double-disk synergy test combined with Etest ESBL (ABBiodisk, Solna, Sweeden) were used as phenotypical reference method. Moreover, twelve strains with genotipically identified β-lactamases were used as controls.
Results: With Etest as the reference for the clinical isolates and the genoptype as the reference for the control strains, the ESBL specific test of the AST-N058 card and UNMIC/ID-62 panels detected the ESBLs with accuracies of 99.49% and 89.39%, respectively. No significant difference between the systems with regard to the control strains was detected. Because of BDXpert system, the Phoenix enhanced its accuracy up to 96.46%. However, the VITEK 2 expert system considered as incoherent the AST results in 7 isolates with ESBL positive tests.
Conclusions: The ESBL test of the VITEK 2 system (AST-N058 card) performed better than Phoenix (UNMIC-ID 62 panel) in detecting the ESBLs produced by clinical isolates of *E. coli* and *K. pneumoniae*. Nevertheless, the action of expert systems put on the same level both automated systems.

P1186 Comparative mutant prevention concentration values for tigecycline and ciprofloxacin against clinical isolates of *Escherichia coli*

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Objectives: The mutant prevention concentration (MPC) defines the antimicrobial drug concentration that blocks the growth of the least susceptible cell from high density bacterial inocula. Tigecycline, a member of a new class of antimicrobials (glycylcylines) has been shown to have in vitro activity against clinical isolates of *E. coli*. We tested *E. coli* isolates by MPC against tigecycline and ciprofloxacin to determine if strains with high ciprofloxacin MPCs would impact on values for tigecycline.
Methods: A total of 24 strains collected from patients with urinary tract infection were studied. For MPC testing, 10^{10} CFUs were applied to Mueller-Hinton agar plates containing doubling drug concentrations of each drug. Following incubation under optimal conditions for 24–48 hours, the lowest concentration preventing growth was recorded as the MPC.
Results: For all strains tested, tigecycline MPC values ranged from 0.5–2 mg/L; 18 strains (75%) having MPCs at 0.5 mg/L and 5 (21%) at 1 mg/L. For ciprofloxacin, MPC values ranged from 0.031 to ⩾32 mg/L; 7 strains (29%) had MPC values of ⩾2 mg/L with 3 strains (12.5%) at ⩾32 mg/L. Tigecycline MPC against the 7 strains with elevated ciprofloxacin MPC values were 0.5–1 mg/L.
Conclusions: Tigecycline MPC values were low against clinical isolates of *E. coli* with 96% of strains having values of 0.05–1 mg/L. As well, high MPC values to ciprofloxacin did not negatively impact on tigecycline. Tigecycline may be a suitable alternative for therapy in patients infected with quinolone resistant *E. coli*.

P1187 The activity of mecillinam against *Escherichia coli* with defined mechanisms of resistance to 3rd generation cephalosporins

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Objectives: Resistance to 3rd generation cephalosporins (3GC) in *E. coli* is an increasing problem across the world with resistance rates in some parts of Europe (including the UK) of more than 15% in bacteraemia isolates. The main cause has been the spread of CTX-M Extended Spectrum Beta-Lactamases (ESBLs) but other enzymes such as plasmid-borne AmpC-type and OXA β-lactamases are being increasingly recognised and in some parts of the world *E. coli* strains with multiple enzymes are becoming more common. Mecillinam is an amidinopenicillin that is available in Europe as the oral pro-drug pivmecillinam, and is stable to many β-lactamases. We aimed to establish the activity of mecillinam against an international collection of *E. coli* with defined, often multiple, mechanisms of resistance to 3GC.
Methods: The isolates studied were 30 *E. coli* from an international collection with known β-lactamase content. Presence of specific β-lactamases has previously been established by PCR and sequencing. The collection includes isolates with TEM, SHV, CTX-M, CMY, AmpC, and OXA enzymes, and isolates with multiple enzymes. Susceptibility testing was performed by the BSAC agar dilution method on IsoSensitest agar with an inoculum of 10^4 cfu/spot.
Results: The susceptibility results are shown in the table. All isolates remained sensitive to mecillinam (breakpoint ⩽8 mg/L) and meropenem irrespective of the presence of β-lactamases. For three isolates that contained 5 or more β-lactamases, isolate 4 (TEM, CTX-M1, CMY-2, OXA-1, OXA-2), isolate 7 (TEM, SHV, CTX-M, CMY, OXA-1, OXA-2), and isolate 8 (TEM, SHV, CTX-M1, CTXM2, OXA-2) the mecillinam MICs were 1, 4, and, 8 mg/L respectively.
Conclusions: With the spread resistance to 3rd generation cephalosporins, it is important to find alternative agents. Unfortunately many strains exhibit multi-resistance to unrelated compounds as seen in this study. However mecillinam retains activity against *E. coli* even in the presence of multiple β-lactamases. It should be further evaluated to establish its clinical utility in the treatment of infections caused by such organisms.

	MIC_{50}	MIC_{90}	% Sensitive
Mecillinam	0.5	4	100
Meropenem	0.03	0.06	100
Cefotaxime	32	>128	18.8
Amoxicillin	>128	>128	0
Co-amoxiclav	>128	>128	0
Piperacillin/tazobactam	16	128	56.3
Ciprofloxacin	8	128	43.8
Nitrofurantoin	16	64	18.8
Trimethoprim	>128	>128	31.3
Gentamicin	0.5	64	56.3

P1188 The in vitro susceptibility of *E. coli* and *P. aeruginosa* from Minsk, Belarus

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Objectives: In this study we assessed the antimicrobial susceptibility of 69 *E. coli* (2 ESBL+ve) and 61 *P. aeruginosa* strains from Minsk, Belarus.
Materials and Methods: The following antimicrobials were utilised; meropenem (MER), amoxicillin (AMOX), co-amoxiclav (Co-A), cefotaxime (CTX), ceftazidime (CAZ), (tigecycline (TGC), sulphamethoxazole/trimethoprim (COT), piperacillin/tazobactam (P/T), gentamicin (GEN), amikacin (AMI) and ciprofloxacin (CIP). MICs were performed using CLSI agar dilution methodology using cation-adjusted Mueller Hinton agar. The percentage resistant using CLSI breakpoints were calculated.
Results: The range, MIC50 and MIC90 (mg/L) are shown on the table below. Using CLSI breakpoints and MIC90 data all *E. coli* strains were susceptible with the exception of AMOX and Co-A. CIP was the most potent agent tested (0.015 mg/L). Levels of resistance in *P. aeruginosa* were much higher with 15–35% of strains being resistant to MER, CAZ, P/T, AMI and CIP. 77% resistance to GEN was observed.

Antibiotic	*E. coli* n = 69				*P. aeruginosa* n = 61			
	range	MIC_{50}	MIC_{90}	% R	range	MIC_{50}	MIC_{90}	% R
Meropenem	⩽0.008–0.12	0.015	0.03	0	0.06–16	4	16	17
Amoxicillin	2–>128	>128	>128	57	NT	NT	NT	NT
Co-amoxiclav	4–>128	32	>128	57	NT	NT	NT	NT
Cefotaxime	0.03–128	0.03	0.12	3	16–>128	32	>128	67
Ceftazidime	0.06–64	0.12	0.5	3	1–>128	4	>128	27
Tigecycline	0.25–0.5	0.25	0.5	0	NT	NT	NT	NT
Sulph/trim	0.12–128	0.03	0.5	0	NT	NT	NT	NT
Pip/taz	1–32	2	16	0	2–>128	16	>128	32
Gentamicin	0.25–>128	1	2	8	1–>128	32	>128	77
Amikacin	0.05–>128	2	4	2	2–>128	16	>128	25
Ciprofloxacin	<0.008–128	0.015	1	9	0.12–>128	32	64	28

NT = not tested.

Conclusion: Rates of resistance in *E. coli* were low with significant resistance occurring only to amoxicillin with and without clavulanate. Two strains were ESBL positive. In contrast resistance in *P. aeruginosa* was high, only 20% were susceptible to gentamicin.

P1189 **Activity of ertapenem and other agents against clinical isolates of Proteeae producing extended-spectrum or AmpC-type β-lactamases**

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Background: *Proteus* spp. and other enterics of the tribe Proteeae (*Morganella morganii* and *Providencia* spp.) are common causes of urinary tract infections and sometimes responsible of other infections (e. g. wound, intra-abdominal and bloodstream infections). Strains with reduced susceptibility to expanded-spectrum cephalosporins due to production of extended-spectrum β-lactamases (ESBLs) or AmpC-type enzymes have emerged in several settings and, in Italy, have a notable epidemiological impact. In this work we investigated the activity of carbapenems and other potentially active agents against a collection of clinical isolates of Proteeae producing a variety of ESBLs or AmpC-type β-lactamases.
Methods: Antibiotic susceptibility was determined by the CLSI agar dilution method or by Etest. Inoculum size effect was determined with 106 vs. 104 CFU/spot). Characterisation of ESBLs or AmpC-type enzymes was carried out by PCR and sequencing. A total of 102 nonreplicated clinical isolates of Proteeae (75 *Proteus mirabilis*, one *Proteus vulgaris*, five *M. morganii*, 15 *Providencia stuartii* and two *Providencia rettgeri*) producing an ESBL (TEM-, CTX-M- or PER-type) or an AmpC enzyme (CMY-2-like) were investigated.
Results: Using a standard inoculum (104 CFU), susceptibility rates were: ertapenem, 100%; piperacillin/tazobactam, 100%; imipenem, 96%; amoxicillin/clavulanate, 33%; ciprofloxacin, 18%; amikacin, 95%; tobramycin, 50%; and gentamicin 23%. Inoculum size variably affected carbapenem susceptibility. Overall, with higher inocula, susceptibility rates decreased to 98% or 42% for ertapenem and imipenem, respectively. Inoculum size effect was overall higher with strains producing TEM-type ESBLs or CMY-2-like AmpC enzymes.
Conclusions: Present results indicate that carbapenems and piperacillin-tazobactam were the most active agents against ESBL- and AmpC-producing Proteeae of clinical origin. Among carbapenems, ertapenem was the most active, and MICs remained usually lower than the breakpoint for susceptibility even in the presence of higher inoculum; imipenem susceptibility was remarkably affected by the inoculum size.
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Antibiotic resistance in Gram-positive organisms

P1190 **Characterisation of Gram-positive conjugative transposons in enterococci from different sources by a multiplex-PCR assay**

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Objectives: Conjugative transposons (CTn) have contributed to the spread of tetracycline (TeR) and erythromycin (ErR) resistance and might influence the adaptation of enterococci to different environments. Diversity of CTn among enterococcal isolates was analysed by a new multiplex PCR assay that includes amplification of specific sequences of known CTn from Gram-positives and others Tn often associated with them.
Methods: We studied 146 *E. faecium* (Efm), 140 *E. faecalis* (Ef), 84 *Enterococcus* spp. from 114 patients of 3 hospitals (H, n = 314 isolates), 20 samples of 3 piggeries (PE, n = 33), 76 poultry retail samples from 2 butcher shops (P, n =167); 7 hospital sewage/river samples (SR, n = 33); and 2 healthy humans (HV, n = 3) from 1996 to 2006. Antibiotic susceptibility and conjugation experiments were performed by standard procedures. Species identification and characterisation of tet (tetM, tetO, tetL, tetS, tetK)/ermB genes was performed by PCR. A battery of multiplex PCR were developed for screening integrases, excisionases or tranposases of Tn916, Tn917; Tn5397, Tn5398, Tn5252, EfcTn1, or Tn5386. Specificity of the method was proved by using specific control strains and sequencing PCR products.
Results: Most isolates were non susceptible to tetracycline (Te, 81%). tet genes were identified among TeR (n = 391/325, 90%) and TeS (n = 34/235, 10%) isolates. Among isolates containing tet we found tetM, tetS and tetL in both *E. faecalis* (100%, 59%, 4%) and *E. faecium* (92%, 80%, 2%). Occurrence of int/xisTn916 or tnpA/tnpRTn917 was slightly higher for *E. faecalis* than for *E. faecium* or *Enterococcus* spp. (49%/ 32%/ 37% or 37% /25%/ 11%, respectively) and spread among H, P and PE isolates. Conversely, tndXTn5397 was more common among *E. faecium* than *E. faecalis* or *Enterococcus* spp. (47%, 6%, 27%, respectively) and mainly confined to P isolates. EfcTn1 was recovered from 2 swine isolates (*E. faecium* and *Enterococcus* spp.). Most of the isolates contained sequences of 2 Tn, mostly Tn916/Tn917. Tn sequences (Tn916, Tn917, Tn5397, Tn5398) were transferred in 58% of the cases.
Conclusion: This method enables to screen CTn and other Tn often associated with them in a high number of strains. Association of particular elements with some species probably reflects differences among bacterial communities exchanges to which they belong. We firstly described EfcTn1 among enterococcal from swine.

P1191 **Molecular epidemiology of vancomycin-resistant enterococci in Belgium, 2003–2006**

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Background: To study the molecular epidemiology of vancomycin-resistant enterococci (VRE) isolated from clinical specimens from hospitalised patients in Belgium during 2003–06.
Methods: VRE were identified on a multipex PCR detecting vanA, B, C1/C2, D, E and G, and on vancomycin MICs. Speciation was done on a duplex PCR detecting ddl genes, and for non-*E. faecalis*/*faecium*, on a sodA PCR and DNA sequencing. MICs to ampicillin, ciprofloxacin, gentamicin, streptomycin, quinupristin/dalfopristin, and linezolid were determined by agar dilution. Presence of the linezolid resistance mutation G2576U was studied by PCR of the 23S rRNA and a subsequent RFLP using NheI. Presence of virulence genes asa1, gelE, cylA, esp, and hyl was detected by a multiplex PCR. Genetic relatedness was examined by PFGE using SmaI, and an MLST analysis on the predominant VRE clones.
Results: Ninety-three VRE were isolated during 2003–06. Of these, 77 (83%) were *E. faecium*, 12 (13%) *E. faecalis*, and 4 (4%) *E. gallinarum*. vanA, the predominant vancomycin resistance gene, was identified in 77 VRE, vanB in 5, vanA+vanB in 1, and vanC1/C2 in 4 isolates, while 6 VRE did not show presence of any van gene. The predominant PFGE clone 54, which constituted 52% of the VRE studied, was an ampicillin- and ciprofloxacin-resistant, vanA-harbouring, hyl-esp-positive *E. faecium*. MLST analysis showed clone 54 to belong to ST17/CC17. The esp gene was present in 84% of the total VRE and in 90% of the *E. faecium* isolates either alone or with hyl, gelE, asa1, cylA. Ampicillin resistance was observed in 86% isolates, high-level gentamicin resistance (MIC > 500 μg/ml) in 38%, high-level streptomycin resistance (MIC > 2000 μg/ml) in 91%, and combined high-level gentamicin and streptomycin resistance in 38% isolates. Among non-*E. faecalis* VRE, resistance to quinupristin/dalfopristin (MIC $\geqslant$4 μg/ml) was 14%. One *E. faecalis* was linezolid-resistant (MIC 16 μg/ml) and RFLP generated two bands confirming presence of the G2576U mutation in 23S rRNA. Three *E. faecium*, of which two belonged to clone 54, were intermediately resistant to linezolid (MIC 4 μg/ml), however, the G2576U mutation was not detected.
Conclusions: Our study highlights the predominance of an ampicillin- and ciprofloxacin-resistant vanA-harbouring, hyl-esp-positive *E. faecium* ST17/CC17 clone among hospital-associated VRE infections in Belgium. The esp virulence gene, a marker for hospital-adapted VRE with an epidemic potential, was identified in majority of the VRE studied here.

P1192 **Molecular epidemiology and antibiotic-resistant genes of *Enterococcus faecalis* causing paediatric infection in Cuba**

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Objetives: The aim of this work was investigate the genetic relatedness and antibiotic resistant determinants in *Enterococcus faecalis* isolates from clinical samples in a paediatric hospital from Cuba.
Methods: A total of 55 *E. faecalis* isolates were recovered from different clinical samples between January 2001/December 2004 from unrelated children admitted in a paediatric hospital in Cuba. Antimicrobial susceptibility was assed by the agar dilution method. Pulsed-field gel electrophoresis (PFGE)-SmaI was performed for assessing the clonal relatedness among the isolates. Presence of the antibiotic resistance genes: erm(B), erm(A), erm(C), mef(A), tet(M), aac(6′)-aph(2"), aph(3’), ant(6), ant(3")(9), aph(2")-id and aph(2")-ic were tested by PCR with specific primers.
Results: A total of 17 different patterns of PFGE were identified among the 55 *E. faecalis* isolates, detecting the same enterococcal clone in different patients. Presence of a single clon for a single patient was only detected in six cases. Particular multi-resistant *E. faecalis* clones were detected in different wards from the paediatric hospital suggesting an intrahospital circulation of the selected clones. aac(6′)-aph(2″) and aph(3′) genes was detected in all HLR-gentamicin and HLR-amikacin isolates, respectively; whereas the ant(6) gene were detected in the 75% of HLR-streptomycin isolates. Most of the isolates contained simultaneously genes for multiple aminoglycoside-modifying enzymes. The erm(B) gene was found in 72% of the erythromycin-resistant strains. Multi-resistance to three or more antibiotic families was observed in 27 isolates which belonged to 13 *E. faecalis* clones.
Conclusion: The circulation of particular clones exhibiting antibiotic resistance within the paediatric hospital and the dissemination of genes among these strains indicated the need of infection control measures and the monitoring of antibiotic susceptibility to prevent the selection or dispersion of resistant strains in the hospital.

P1193 **Phenotypic and genotypic characterisation of antimicrobial resistance in faecal enterococci of healthy humans and animals**

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Objective: To characterise the phenotypes and genes implicated in antimicrobial resistance in faecal enterococci of healthy humans and animals.
Methods: 171 faecal samples of healthy animals (66 pigs, 22 pets and 15 bulls) and humans (50 children under two years old and 18 adults) were seeded onto mEnterococcus agar plates. Two colonies per sample were selected and identified. Susceptibility testing to 10 antimicrobials were carried out by agar dilution method and aminoglycoside, glycopeptide, macrolide and tetracycline resistance genes were studied by PCR and sequencing.
Results: A total of 133 *E. faecalis*, 63 *E. faecium*, 55 *E. hirae*, 4 *E. casseliflavus*, 4 *E. gallinarum*, 4 *E. avium*, 3 *E. durans* and 7 *Enterococcus* spp. were recovered. *E. faecalis* was found in 72% of the isolates from human origin, and this species in addition to *E. hirae* were the most prevalent ones among pig samples (43 and 37%, respectively). *E. faecium* was found in 57% of isolates from pets and bulls. The percentages of antibiotic resistance (found in isolates from pigs/pets and bulls/human samples) were as follows: tetracycline (93/66/54%), erythromycin (81/23/29%), ciprofloxacin (18/23/14%), penicillin (5/6/0%), vancomycin MIC > 8 mg/L (0.9/2/4%) and high-level resistance (HLR) to streptomycin (44/9/12%), gentamicin (6/6/5%) and kanamycin (37/9/14%). None of the 273 enterococci showed ampicillin- or teicoplanin-resistance, whereas the highest resistance were found to tetracycline (72%) and erythromycin (49%). One *E. faecalis* isolate of porcine origin showed a VanB phenotype characterised by acquired inducible resistance to vancomycin and susceptibility to teicoplanin (MICs of 8 mg/L and ⩽0.5 mg/L, respectively). The vanC gene was found in the six *E. gallinarum* and *E. casseliflavus* isolates recovered from human and bulls. The ant(6)-Ia, aac(6′)-aph(2″) and aph(3′)-III genes were demonstrated in most of the isolates with HLR to streptomycin, gentamicin and kanamycin, respectively. The ermB and tet(M) genes were detected in 95% and 92% of erythromycin- and tetracycline-resistant isolates, respectively.
Conclusions: Differences in antimicrobial resistance percentages were observed among faecal enterococci of different origins, being those recovered from pigs the most resistant to tetracycline, erythromycin and streptomycin. The normal microbiota of healthy humans and animals represents a considerable reservoir of antimicrobial resistance genes.

P1194 **MRSA of clonal lineage ST398 in clinical isolates in Austria**

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Objectives: Traditionally, meticillin-resistant *Staphylococcus aureus* (MRSA) is a problem of infection control in hospitals, but since the detection of MRSA in the community and in animals it has received a new focus. In the present study we aimed at identifying MRSA strains of clonal lineage MLST 398 in clinical isolates and at looking for possible associations with animals as well as the magnitude of clinical relevance.
Methods: We investigated all MRSA-isolates detected between January 2006 and November 2007 in Upper Austria (one of the nine federal provinces in Austria) by PFGE, spa-typing and MLST typing in clinical isolates. The resistance of these strains to ciprofloxacin, clindamycin (CLI), doxycycline (DOX), erythromycin (ERY), fosfomycin, fusidic acid, gentamicin, moxifloxacin, mupirocin, rifampicin and vancomycin was determined using the Etest® method. Furthermore demographic information and data from patient histories were collected and interpreted by infection control teams in hospitals as well as physicians in outpatient care.
Results: During a period of 2 years we identified 756 patients colonised/infected with MRSA. In 12 patients (8 male, 4 female; median age 58 years, range 1–81) we identified MRSA of clonal lineage ST398 and of spa types t011, t034 and t2346. None of the strains harboured the PVL gene. The isolates were obtained from chronic wounds (6), eye (1) or nose/throat/trachea (3). 8 of these patients were colonised, 2 were infected with MRSA ST398, and of 2 the status was unknown. 6 isolates were resistant to CLI, ERY and DOX, 5 isolates were resistant to only DOX, and 1 isolate was fully susceptible. Epidemiological links to pig farming could be confirmed in 7 patients, and links to farming in 4 patients.
Conclusions: Similar to other countries, also in Austria, MRSA of clonal lineage ST398 is associated with animal contact. There are obvious signs that transmission of MRSA between humans and animals, especially pigs, occurred. We only investigated clinical isolates, however, we presume that MRSA colonisation might be more common in the general population. Further investigations to determine the circumstances and ramifications of transmission from animals to humans need to be performed.

P1195 **Prevalence of pig-associated meticillin-resistant *Staphylococcus aureus* spa types in the German EUREGIO Twente/Münsterland**

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Objectives: Recent studies indicated that pig-farming is a risk factor for MRSA-colonisation in patients admitted to Dutch hospitals. Pig-related MRSA mostly belonged to *Staphylococcus aureus* protein A (spa) type t011 and its close relatives (t034, t108). Until now, MRSA t011 were rarely found among patients in German hospitals. Since in the EUREGIO Twente/Münsterland pig-farming has a high socio-economic impact, we raised the question how many patients carry MRSA t011 in the German

part of the EUREGIO and asked whether these patients can be identified by screening for classical nosocomial MRSA risk factors.
Methods: In November 2006, all patients admitted to 39 hospitals in the German part of the EUREGIO Twente/Münsterland were screened by nasal swabbing. Risk factors for MRSA carriage were assessed including previous MRSA carriage, contact to MRSA patients, antibiotic prescription, hospitalisation during the past six months, direct transfer from a nursing home or from hospitals, presence of catheters, skin lesions or combustions, chronic need of healthcare and haemodialysis.
All MRSA were spa typed and clustered in spa clonal complexes (spa-CCs) using the Based Upon repeat pattern (BURP) algorithm. Chi-square test or Fisher's exact test were used for analysing differences in the distribution of risk factors and patient population. A p-value <0.05 was considered statistically significant.
Results: Screening yielded a MRSA prevalence of 1.7 MRSA/100 screenings. Among 354 MRSA, 62 isolates clustered in spa-CC011 (17.8%) comprising t011, t034, t108, t567, t1451, and t2011. 286 MRSA (82.2%) clustered in other spa-CCs (spa-CCnon011). 55% of all spa-CC011 and 26.6% of all spa-CCnon011 patients lacked any of the risk factors assessed ($p < 0.001$). Chronic need of healthcare ($p < 0.001$), transfer from nursing homes ($p < 0.001$), and catheters ($p = 0.005$) were less frequent among spa-CC011 patients. spa-CC011 was associated to patients admitted to surgical departments ($p = 0.009$) and neurology or psychiatry ($p = 0.02$), but was underrepresented in internal medicine ($p = 0.01$) and geriatric care ($p = 0.009$).
Conclusions: MRSA spa-CC011 are prevalent in most hospitals in the German part of the EUREGIO. Among these, the predominant spa type was t011. Patients found to carry MRSA spa-CC011 were less frequently associated with classical nosocomial risk factors compared to other MRSA patients. Further investigation is needed to assess pig-farming as a potential risk factor for MRSA in the EUREGIO.

P1196 Multicentre in vitro evaluation of Gram-positive micro-organisms in the Netherlands for daptomycin compared with other antibiotics

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Background: Daptomycin (DA) is a new lipopeptide recently approved in The Netherlands (NL). In a multi-centre study, we aimed to evaluate the in vitro antibacterial activity of daptomycin and other antibiotics against various Gram-positive micro-organisms.
Methods: 25 laboratories equally distributed throughout the Netherlands were asked to participate in the study. Each lab was asked to collect up to 90 consecutive isolates from clinical samples (patient >72 h in hospital) during a 3 month period (june-sept 2007), 25 *S. aureus* (SA), 25 Coagulase Negative Staphylococci (CNS), 20 enterococci (EN) and 20 *Streptococcus* spp. other then pneumococci (ST). All isolates were required to be from blood, pus, sites normally sterile or wounds; for CNS at least two cultures needed to be positive. A maximum of one isolate per species per patient was allowed. Isolates were identified by participating laboratories using their own standard identification technique. MIC's were determined using Etest on site for DA, vancomycin VA), teicoplanin (TE), linezolid, gentamicin, erythromycin or clarithromycin, penicillin and cefotaxim (ST), ampicillin (EC), moxifloxacin and rifampicin (staphylococci). BHI agar with 2.0 McFarland and 48 h incubation was used for VA and TE, 0.5 McF on Mueller-Hinton and 24 h incubation for the others. The cefoxitin disk method was applied to staphylococci. Control ATCC strains were included. Afterwards, strains were collected by the central lab for further analysis. All strains were retested to VA, TE and DA. EUCAST criteria were used for interpretation. Data were analysed using WHONET 5.4.
Results: 1709 strains were collected, 525 SA, 427 CNS, 394 EC, and 363 ST. MIC50's for daptomycin were 0.38, 0.5, 1.5 and 0.125 mg/L and MIC90's 0.75, 1, 3 0.5 mg/L, respectively. 36 CNS had MICs of >1 mg/L for DA. 67 strains were categorised as VISE's based on an increased MIC (TE > 8 mg/L). There was a significant correlation between increased MIC for DA and VA (r=0.42; $p < 0.0001$) and TE (r=0.34; $p < 0.0001$) respectively. VA and TE resistance in enterococci was not observed. Four SA showed a slightly increased MIC (3× MIC=1.5 mg/L; 1× 3 mg/L)
Conclusions: Daptomycin showed excellent activity to these clinical isolates. However, staphylococci with decreased susceptibility to glycopeptides showed higher MICs to daptomycin too, possibly limiting the use of daptomycin in this small subgroup.

P1197 Clonal spread of linezolid and teicoplanin-resistant *Staphylococcus haemolyticus* in intensive care unit patients in a tertiary hospital

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Objectives: Following the observation of several cases of infections caused by isolates of linezolid and teicoplanin-resistant *S. haemolyticus* (LTRSH) we decided to investigate the epidemiology and clonality of these isolates, and to determine the molecular mechanism of resistance to linezolid.
Methods: Medical records of all patients with LTRSH in the last three years were reviewed. Antimicrobial susceptibility testing was performed with Wider panels (Soria-Melguizo, Madrid, Spain) and minimun inhibition contentrations (MIC) were performed by the Etest method for linezolid, teicoplanin, vancomycin, tigecycline and daptomycin. Molecular characterisation of LTRSH isolates was performed by pulsed-field gel electrophoresis (PFGE) after SmaI digestion. A 420bp Domain V region of the 23S gene was amplified for each isolate and the products were digested with NheI restriction enzyme for detecting the G2576T mutation, which has been associated with linezolid resistance.
Results: Between October 2005 and March 2007 we recovered 22 clinical isolates of TLRSH from 15 patients. The mean age was 56 years (SD 11.75) and 66.7% were male. All patients were diagnosed of LTRSH infections while they were in ICU. Fourteen cases were considered clinical infections: 11 catheter-related bacteraemia and 4 surgical infections. Eleven patients underwent abdominal surgery, including four patients with liver transplantation. All isolates were resistant to meticillin, erythromicin, clindamicin, levofloxacin, rifampicin, trimethoprim-sulfamethoxazole, teicoplanin (range 24–128 mg/L) and linezolid (range 32 to 128 mg/L) and susceptible to vancomycin, tigecycline and daptomycin. PFGE analysis indicated that all isolates belonged to a single clonal group. All linezolid-resistant isolates had the same 23S rRNA mutation in domain V, G2576U, and the incomplete digestion of NheI indicated that not all copies of rRNA genes carried the mutation.
Conclusion: This report documents the spread and persistence of a single clone of multidrug-resistant *S. haemolyticus*, including resistance to linezolid and teicoplanin, in ICU patients. This study confirmed previous reports regarding the importance of the G2576T mutation associated to linezolid resistance in *Enterococcus faecium*, *Staphylococcus epidermidis* and *S. haemolyticus*. The emergence of LTRSH in hospitals has important clinical and epidemiological implications.

P1198 Investigation of the genetic basis of erythromycin resistance in *Staphylococcus aureus*, including MRSA, from Pakistan

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Objectives: Erythromycin (ERY) is used extensively in clinical practice in Pakistan for skin and throat infections, but little surveillance work has been done to assess the extent of resistance amongst common pathogens. Similarly, there is a paucity of information about the MRSA clones circulating. The aim of this study was to determine the prevalence and mechanisms of ERY resistance amongst *S. aureus* from specimens being processed at a diagnostic laboratory in the North West of Pakistan. Also, to see if there were differences in the extent of ERY resistance between meticillin sensitive (MSSA) and resistant (MRSA) strains.
Methods: Sequential isolates of *S. aureus* were collected from wounds and burn/skin infections of in- and out-patients at a tertiary care hospital

in Peshawar. Isolates were identified by standard methods, including PCR for mecA/nuc genes to confirm MRSA, which were also ribotyped. MIC profiles for commonly used antibiotics were determined using CLSI criteria. Presence of erm(A), erm(B), erm(C) and msr(A) genes was determined by PCR.

Results: Of 133 isolates of *S. aureus* collected, 59 (44.4%) were resistant to ERY, with MIC values ranging from 8 to >1024 mg/L. Just 3.3% (n = 3) of the 70 MSSA were resistant, while in contrast, of the 63 MRSA strains, 41.3% (n = 35) were ERY-R. The most common resistance gene detected amongst the ERY-R strains was erm(A) (44.1%), followed by erm(C) (42.3%), msr(A) (8.4%) and erm(B) (5%). No strain carried multiple genes. Eight ribotypes (A to H) of MRSA were present: erm(A) was found in ribotypes A, B and C, erm(C) was found in ribotypes D, E and F. The erm(B) +ve strains were restricted to ribotype G, and msrA to ribotype H. Overall, ERY-R strains of MRSA were generally co-resistant to ciprofloxacin (86.8%), gentamicin (86.8%) and tetracycline (83.6%). However, resistance amongst such strains to chloramphenicol and fusidic acid was much lower (20% and 16.3% respectively).

Conclusion: ERY-R was surprisingly rare amongst skin isolates of MSSA from this area, and thus this antibiotic may still be useful for treating infections if MSSA status is confirmed. Amongst the MRSA, ERY-R is common, mediated mostly by erm(A) and erm(C), and the resistance genes map to the clonal lineages. Where the skin infection is due to an MRSA, topical fusidic acid may still have utility in this area of Pakistan.

P1199 Distribution of macrolide resistance genotypes and phenotypes among *Staphylococcus aureus* and coagulase-negative staphylococci, Italy

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One-hundred macrolide-resistant staphylococcal isolates from clinically relevant infections in Italy during a 19-months period were studied to determine macrolide resistance genotypes and phenotypes. Macrolide resistance phenotypes were performed by using the triple-disk induction test with 15-μg ERY disk, 2-μg CLI disk, and 15-μg TEL disk. Four distinct resistance phenotypes were observed among 100 erythromycin-resistant isolates: the cMLSB phenotype (24 isolates); the iMLSB phenotype (41 isolates); the MS phenotype (3 isolates); and the iMTS phenotype (erythromycin-induced telithromycin resistance) (32 isolates). All isolates with MS and iMTS phenotypes were msrA-positive, whilst all isolates with MLSB phenotype were erm-positive. ermC gene predominated within erythromycin-resistant *S. aureus* isolates with iMLSB phenotype, while ermA gene was present in all but one *S. aureus* isolates with cMLSB phenotype. The msrA gene was found in only one *S. aureus* isolate. Among erythromycin-resistant CoNS isolates half of the strains showed the iMTS or MS/msrA association. ermC gene was present in all but 2 erm-positive CoNS isolates. Among 76 erythromycin-resistant isolates studied by PFGE an high genetic heterogeneity was observed, with both independent acquisition of macrolide resistance genes and spread of specific resistant clones as mechanisms responsible for occurrence of macrolide resistance. To our knowledge, it is the first report on the prevalence of the erythromycin resistance phenotypes and genotypes among staphylococcal clinical isolates in Italy.

P1200 Distribution of mefE and ermB in macrolide-resistant *Streptococcus pneumoniae* isolated from nasopharynx of healthy children

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Objectives: The vast use of macrolides has been connected with increased prevalence of macrolide-resistant strains of pneumococci. In many European countries, the rate of pneumococcal resistance to macrolides has exceeded the level of resistance to penicillin G. More than 20% rates of resistance to erythromycin are reported in France, Spain, Poland, Greece, and Portugal. Macrolide resistance is mediated by two main mechanisms in pneumococci. The most common mechanism is mediated by methylase encoded by the erm(B) gene. Ribosome methylation causes reduced binding of and coresistance to macrolide, lincosamide, and streptogramin B (MLS) antibiotics. In streptococci MLS resistance can be expressed either constitutively (cMLS phenotype) or inducibly (iMLS phenotype). Macrolide efflux mechanism is mediated by an efflux pump encoded by mef(E) gene. It is associated with M phenotype characterised by resistance to 14- and 15-membered macrolides only. We selected erythromycin-resistant strains of *S. pneumoniae* isolated from nasopharynx of healthy children and tested them for resistance phenotype and analysed for genes responsible for macrolide resistance.

Methods: 113 erythromycin-resistant *S. pneumoniae* strains were tested for resistance phenotype by erythromycin-clindamycin double-disk (ECDD) test. The presence of erythromycin resistance genes was investigated by PCR. Primer pairs specific for detection of erm(B) and mef(E) and amplification and electrophoresis of PCR products were carried out by Sutcliffe et al. (AAC 1996).

Results: Erythromycin-resistant *S. pneumoniae* strains belonged mainly to 19F (55%) and 6B (37%) serotypes and remaining single strains belonged to 23F, 14, 15B, and 23A. 74% pneumococcal strains were also non-susceptible to penicillin. All 113 erythromycin-resistant strains tested using the ECDD assay were assigned to the cMLS phenotype. iMLS and M phenotypes were not found by this method. All strains had the erm(B) gene and 29 (25.7%) strains showed both erm(B) and mef(E) genes. There were no correlation between coexisting erm(B) and mef(E) and serotypes or resistance to other antibiotics, including penicillin.

Conclusion: The predominance among erythromycin-resistant pneumococci of isolates carrying the erm(B) gene over those carrying the mef(E) is consistent with result of other studies from European countries. Prevalence of isolates with combined mef(E) and erm(B) genotypes is increasing particularly in children.

P1201 Population structure of erythromycin-resistant *Streptococcus pneumoniae* invasive isolates recovered in a Spanish hospital in Madrid

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Objectives: To analyse the population structure of erythromycin resistant *Streptococcus pneumoniae* isolates and further characterisation of mef-positive isolates recovered from blood cultures in a hospital in Madrid. Prevalence of associated resistance phenotypes and genetic determinants were also screened as well as the antimicrobial co-resistances.

Methods: Among all 334 *S. pneumoniae* invasive isolates recovered from 2000 to 2006 in our hospital a PCR assay was performed to identify erythromycin resistance genes [erm(B) or mef]. A multiplex-PCR was designed to distinguish between mef(A) and mef(E). Susceptibility testing was performed by the standard microdilution technique (CLSI) and resistance phenotypes by the double disk diffusion assay. Population structure was studied by serotyping and SmaI-PFGE among erythromycin resistant isolates and also by MLST within mef-positive isolates.

Results: A total of 68 erythromycin resistant isolates (20.3%) were obtained, with no significant (Mantel Haenszel test) trend to decrease over the studied period. Overall resistance values among these isolates were: penicillin, 61.7%; cefotaxime, 8.8%; clindamycin, 61.7%; tetracycline, 78%; and 1.5%, levofloxacin. Resistance gene distribution were: erm(B), 75.0%; erm(B)+mef(E), 16.2%; mef(E), 7.3%, and mef(A), 1.5%. The iMLSB and cMLSB phenotypes were observed in 75.0% and 16.2% of isolates, respectively, whereas the M phenotype was observed in 8.8%. Serotype 14 (20.5%), 6B (13.2%), 19A (13.2%), 19F (7.3%), 6A (5.8%), and 4 (5.8%) were predominant. Most isolates belonging to the same serotype showed different PFGE pattern. Spain^{6B-2}, Sweden^{15A-25} and Tenesse$^{14-18}$ international multi-resistant clones were found within the erm(B)+mef(E) positive isolates. England$^{14-9}$ was represented by two isolates, one carrying the mef(A) gene and the other one the mef(E) gene. Interestingly, Spain^{9V-3} clone

were both represented in two mef(E) positive isolates and additionally in three erm(B)+mef(E) positive isolates.
Conclusions: Erythromycin resistance in our invasive isolates remains high mainly due to the presence of erm(B) gene. Nevertheless a high proportion of these isolates also harboured the mef gene endowing the iMLSB phenotype. A polyclonal population structure was found in our mef positive *S. pneumoniae* isolates which included international multirresistant clones.

P1202 Conjugative transposon Tn5253 of *Streptococcus pneumoniae*

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Objectives: The conjugative transposon (CT) Tn5253, also called (cat tet)6001, is a genetic element which integrates at a specific site into the chromosome of *Streptococcus pneumoniae*. Tn5253 was originally found in *S. pneumoniae* strain BM6001, a serogroup 19 clinical isolate resistant to chloramphenicol and tetracycline. Tn5253 was found to carry resistance genes tet(M) and cat, and could be trasferred by conjugation to the chromosomes of other pneumococcal strains and of other bacterial species including *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Streptococcus gordonii*, and *Enterococcus faecalis*. Aim of this study was to obtain the complete nucleotide sequence of Tn5253, as a contribution to our understanding of the resistance-carrying mobile genetic elements that are found in the genomes of *S. pneumoniae* isolates.
Methods: The complete nucleotide sequence of Tn5253 was obtained by using as a template different PCR fragments (size varies between 0.3 to 22 kb).
Results: Sequence analysis showed that Tn5253 is 64,513 bp in size, with 78 ORFs. Tn5253 was also found to contain two distinct genetic elements integrated: (i) a copy of Tn5251, of the Tn916-Tn1545 family of tet(M)-carrying CT, and (ii) a copy of pC194, a cat-containing plasmid of *Staphylococcus aureus*. Tn5251 was found to be 18,039 bp in size and 99% identical to Tn916 of *Enterococcus faecalis*. The copy of pC194 found in Tn5253 (called pC1945253) was 2816 bp in size and, compared to pC194, had a deletion of 93 bp involving the plasmid replication origin. Conclusion.
These data represent the basis for a detatiled analysis of the repertoire of mobile genetic elements in *Streptococcus pneumoniae*, and for a functional analysis of the conjugation properties of this element.

P1203 Distribution of the macrolide resistance genes mefA, mefE, and erm in *S. pneumoniae* and *S. pyogenes* in Germany from 2005 to 2006

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Objectives: *Streptococcus pneumoniae* is the major cause for many childhood diseases like respiratory tract infections, acute otitis media and community-aquired pneumonia. Resistance of S.pneumonia and *S. pyogenes* to macrolide antibiotics has become an increasing problem worldwide due to the spread of the resistance genes mefA, mefE and ermB. Both mef genes encode an efflux pump, resulting in low level resistance to macrolides (M-phenotype). The sequences of mefA and mefE are almost identical, but the genes are carried on different genetic elements: Tn1207.1 and MEGA respectively, that might give rise to differences in clonal spread. ErmB encodes an rRNA methylase which modifies the macrolide binding site in the ribosome, thus giving rise to high level resistance (MLSB-phenotype). We investigated the macrolide resistance markers in Germany (2005–2006), with special emphasis on the distribution of mefA and mefE.
Methods: *S. pneumoniae* and *S. pyogenes* isolates were collected between 2005 and 2006 as part of the national surveillance programs of the German National Reference Centre for Streptococci (GNRCS). Clarithromycin MICs were determined with the microdilution method according to the CLSI criteria. A multiplex PCR was performed to distinguish between mefA and mefE and a separate PCR was used to detect the ermB gene. Multilocus sequence typing (MLST) was performed according to standard methods.
Results: Between 2005 and 2006 the GNCRS received 2065 *S. pneumoniae* and 330 *S. pyogenes* isolates from children and adults from all over Germany. A total of 481 *S. pneumoniae* and 26 *S. pyogenes* isolates were macrolide resistant and of 446 and 20 of these isolates the resistance markers were determined. Among the *S. pneumoniae* isolates the main resistance marker was mefA (55%), followed by ermB (26%) and mefE (11%). 4% were mefA+ErmB, 4% mefE+ermB and 0.4% carried neither mef nor ErmB. Among the *S. pyogenes* isolates 45% were mefA and 55% erm. MLST analysis indicated a clonal dissimination of both mefA and mefE determinants.
Conclusion: mefA is the most common macrolide resistance marker in *S. pneumoniae* and *S. pyogenes* in Germany. This is in contrast to countries like Spain, Canada and the USA, where mefE is far more prominent.

P1204 Analysis of structural alterations in the regulatory region of the erm(TR) gene of *Streptococcus agalactiae* with constitutively expressed MLSB resistance

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Objectives: The methylases conferring MLSB resistance are encoded by erm(TR) and erm(B) in *Streptococcus agalactiae* (GBS). The regulatory region of the erm(TR) gene consists of two leader peptides (LP1 and LP2) containing a number of inverted repeats that can act in attenuator regulation, analogous to that found in erm(C). Little has been published regarding the regulation in GBS of erm(TR). The purpose was to study the regulatory region in GBS clinical isolates harbouring constitutively expressed erm(TR) gene.
Methods: Antimicrobial susceptibility testing to MLSB antibiotics and identification of resistance phenotype were performed by broth microdilution (CLSI guidelines) and triple disk diffusion methodology, respectively. Ten isolates, with constitutively expressed MLSB resistance encoded by erm(TR), were selected among 141 MLSB resistant GBS collected from vaginal swabs obtained from 2002 to 2004 by our Clinical Laboratory (Hospital Lozano Blesa, Zaragoza, Spain). The erm(TR) regulatory region of these isolates were amplified from chromosomal DNA. Therefore, we determined the nucleotide sequences and deduced amino acid sequences of erm(TR) regulatory region. Pulsed-field gel electrophoresis (PFGE) analysis was performed on the 10 GBS isolates following Sma1 digestion.
Results: GBS isolates selected expressed a resistance cMLSB pattern characterised by high resistance to clindamycin (range >64 mg/L) and low resistance to erythromycin (range 8–16 mg/L). Sequencing of the attenuator region upstream erm(TR) showed six different point mutations (C57U) (G98A) (A137C) (C170U) (G205A) (G205T) and only one 863 bp insertion were identified. Most of the changes were found in the ORFs of the second leader peptide (LP2), mainly due to the mutation (A137C). One of the amplification product was greater than expected and carried an insertion of 863 bp in position 121 of LP2. Sequencing of the insertion fragment showed 98% identity with insertion sequence IS1381. Analysis by PFGE revealed that three isolates were closely related (homology >80%).
Conclusions: This study showed that mutations and insertions in the regulatory regions of erm(TR) can be correlated to constitutive MSLB expression due to the destabilisation of the stem-loop that would lead to the translation of the erm(TR) gene and results in increased or constitutive expression of erm(TR) gene.

P1205 Molecular epidemiology and macrolide resistance traits of *Streptococcus agalactiae* and *Streptococcus uberis* from bovine mastitis

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Objectives: To evaluate the clonality and resistance patterns against macrolide and tetracycline of *Streptococcus agalactiae* (GBS) and of *Streptococcus uberis* (SU), both important contagious and environmental pathogens in bovine mastitis, in order to formulate effective mastitis control programs for dairy herds.

Methods: A total of 60 GBS and 30 SU field isolates were recovered among 459 milk samples from mastitis quarters of 377 bovines in 12 Portuguese dairy farms, during a national survey performed in 2002–03, and were characterised by pulsed field gel electrophoresis (PFGE) with computer-assisted DNA-band analysis. GBS and SU representative isolates of different PFGE sub-types were analysed by multilocus sequence typing (MLST). Resistance against macrolides (erythromycin-E), lincosamides (pirlimycin-PRL) and tetracycline-T and the macrolide-lincosamide resistance phenotypes (M/cMLS/iMLS) were evaluated by disk diffusion. The macrolide and tetracycline resistance genes mef(A), erm(A), erm(B), tet(M), tet(O), tet(W), tet(L), tet(Q), tet(K), tet(S) were searched for by PCR among isolates of both species.

Results: A total of 10 PFGE clusters comprised 77% of the GBS and four PFGE clusters comprised 53% of the SU and almost all were found to be herd-specific. The GBS were clustered in ST-2 and ST-61 lineages previously described among human isolates of GBS (according to the *S. agalactiae* MLST database), while the SU isolates were included into 11 novel lineages (seven of which consist of new alleles) clustered into three clonal complexes, ST-143, ST-86 and ST-5, known to include bovine isolates from several geographic locations. Co-resistance to E and T was found in both GBS (17%) and SU (27%) belonging to several clones or lineages and most GBS were cMLS erm(B)$^{+}$/tet(O)$^{+}$/tet(K)$^{+}$ while most SU were cMLS-erm(B)$^{+}$/tet(O)$^{+}$. SU isolates susceptible to E and resistant to PRL and T carried the tet(S).

Conclusions: The results suggested contagious and environmental sources of SU infections and extensive cow-to-cow transmission of GBS. The detection of the same macrolide and tetracycline resistance determinants among isolates of both species suggests vertical and horizontal transmission of these genes. A same phenotype of susceptibility to macrolides and resistance to lincosamides, previously detected in GBS of human origin – LSA phenotype, was found in SU bovine isolates.

P1206 mef(E) is present in *Streptococcus pyogenes* and can be transferred by conjugation

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Objectives: Efflux transport system, associated with the mef gene, confers resistance to 14- and 15-membered macrolides (M phenotype) in streptococci. In *S. pyogenes*, mef(A) was found to be carried by conjugative genetic elements, whereas a genetic element carrying mef(E) in *S. pyogenes* has not been described. Among a global collection of *S. pyogenes* clinical isolates, strains carrying mef(E) were identified. The aim of the study was to characterise the element carrying mef(E) in *S. pyogenes*.

Methods: Among 17 *S. pyogenes* isolates which were positive for the presence of mef(E), one isolate from the United States (MB56SPYO9) was examined: PCR assay and sequencing of mef, PCR mapping targeting mega, MLST and emm typing were performed. Conjugation experiments were carried out by using both *S. pyogenes* and *S. pneumoniae*, as recipient strains. Transconjugants were analysed by PFGE followed by hybridisation with mef(E) probe.

Results: In *S. pyogenes* MB56SPYO9 belonging to sequence type ST36 and emm type 12, the presence of mef(E) was confirmed by sequencing. By PCR mapping targeting mega, the fragments obtained were of the same size as those obtained with a control strain. This indicates the presence of mef(E) in a mega element. By conjugation, the erythromycin resistance was transferred from the donor MB56SPYO9 to both *S. pyogenes* and to *S. pneumoniae* recipient strains. SmaI-restricted genomic DNA from the donor, the recipients, and the transconjugants were examined by PFGE. Analysis of the profiles showed the appearance of a novel band in the transconjugants, suggesting the acquisition of an approximately 50 kb DNA fragment by conjugation. Hybridisation assay by using mef(E) probe revealed that this fragment included mef(E).

Conclusions: In *S. pyogenes*, mef(E) is carried by mega and is included in a large conjugative genetic element. Further investigations will be done to characterise this element.

P1207 Phenotypic and genotypic characterisation of group A *Streptococcus* clinical isolates: antimicrobial resistance patterns, macrolide-resistance genes and emm typing

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Objectives: The aim of the present study was to characterise phenotypically and genotypically clinical isolates of group A streptococci (GAS) from a paediatric population in our geographic region

Methods: A total of 1160 GAS clinical isolates were collected during 1/2003–12/2006. Susceptibility testing was performed using standard disk diffusion and Etest methods. Mechanism of macrolide resistance was phenotypically determined using double-diffusion disk test. The presence of mefA, ermA and ermB genes were detected by PCR. emm types were identified by amplification and sequencing.

Results: The average rates of resistance to erythromycin, clindamycin, azithromycin, tetracycline and chloramphenicol were 14.9%, 0.9%, 14.9%, 11.46%, 0.6%, respectively. A significant increase in macrolide resistance was noted from 12.1% in 2003 to 18.8% in 2006 ($p=0.02$). None of the isolates exhibited resistance to penicillin, ceftriaxone, rifampicin, or ciprofloxacin. Among the erythromycin resistant isolates, 93 (53.7%) expressed the M phenotype, 70 (40.5%) the iMLSB and 10 (5.8%) the cMLSB phenotype. Overall, 93 (53.7%) of the isolates harboured the mefA gene, 70 (40.4%) the ermA and 10 (5.8%) the ermB. The most prevalent emm types were 12, 77, 4 among macrolide resistant and 1, 12, 28 among macrolide susceptible isolates. emm types 77, 4, 12, 28 accounted for 75% of invasive isolates and emm types 12, 1, 4, 28, 77 accounted for 75% of non invasive isolates. emm type 77 was found significantly more frequent among invasive isolates ($p=0.02$). GAS vaccine (in clinical trials) encompasses almost 80% of emm-types detected in our study.

Conclusions: Macrolide resistance rates are increasing among GAS in our region. The predominant determinants conferring resistance to macrolides were the mefA and ermA genes. The emm types included in GAS vaccine cover 80% of streptococcal isolates causing infections in Greece. Continuing surveillance studies are needed to determine the stability of the trends reported above and to guide therapy and development of vaccine strategies.

P1208 Decrease in macrolide resistance and clonal instability among *Streptococcus pyogenes* in spite of increased macrolide consumption

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Objectives: Although macrolide resistance has remained stable in Portugal during 1998–2003 (27%), a rapid inversion of the dominant phenotypes was noted, with a sharp decrease in the proportion of isolates presenting the MLSB phenotype and a concomitant increase in the isolates presenting the M phenotype. To gain further insights into the changes in overall macrolide resistance, in fluctuations of macrolide resistance phenotypes and in the clonal structure of the macrolide resistant population, we undertook the phenotypic and molecular characterisation of 156 macrolide resistant GAS isolated in 2004–2006.

Methods: Antimicrobial susceptibility testing and macrolide resistance phenotype were performed by disk diffusion. All the macrolide-resistant isolates were further characterised. Macrolide and tetracycline resistance genotypes were determined by PCR. A combination of T typing, emm typing, pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) was used. The Bionumerics software was used to create UPGMA dendrograms of SmaI/Cfr9I fragment patterns. The Dice similarity coefficient was used with optimisation and position tolerance settings of 1.0 and 1.5, respectively. PFGE clusters included isolates with ⩾80% relatedness on the dendrograms. MLST analysis was performed on representatives of each lineage.
Results: During the three years of the study, the overall rate of erythromycin resistance was lower than the one documented previously, and a decrease in the prevalence of isolates presenting the M phenotype was also noted. PFGE and MLST were highly concordant and identified eight major lineages, containing 139 isolates (89%). Molecular characterisation revealed a very diverse population. Some of the PFGE clusters identified had already been found, but others were described for the first time in Portugal.
Conclusion: A decline in macrolide resistance in *S. pyogenes* was noted, in spite of high macrolide use, and these changes were accompanied by clonal variations. The emergence of clones not detected previously, the change in the prevalence of the clones already described or even the disappearance of some clones resulted in marked differences in the clonal composition of the population of macrolide-resistant GAS in Portugal.

P1209 Emergence of moxifloxacin resistance in Swedish epidemic *Clostridium difficile* isolates in the era of the global NAP1/027 epidemic

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Objectives: In recent years global outbreaks of *Clostridium difficile* associated diarrhoea (CDAD) in the western world involve the epidemic strain of *C. difficile* characterised as PCR ribotype 027 and PFGE type NAP1. Increased infection rates and more severe cases of diarrhoea have featured these epidemics. Use of modern fluoroquinolones (moxifloxacin/gatifloxacin) has been thought to be the epidemic trigger selecting moxifloxacin resistance NAP1/027 isolates. In Sweden the epidemic strain SE17 (Serogroup C, PCR ribotype 012) has been dominant in nosocomial clustering. Retrospectively only three historical isolates of NAP1/027 have yet been found in Sweden without increased morbidity or epidemic clustering. Prior to 2004 both these Swedish NAP1/027 strains and the SE17 have been fully susceptible to moxifloxacin as opposed to the epidemic NAP1/027 being resistant. Hence we used moxifloxacin resistance as a surveillance marker when testing clinical isolates 2004 (n = 300) and 2007 (n = 300) for any emergence of moxifloxacin resistance.
Aim: To determine whether moxifloxacin resistance is a useful general marker of epidemic isolates or exclusively a screening tool of NAP1/027.
Material and Methods: MIC determination. Anaerobically cultured isolates were suspended in nutrient broth (Oxoid) to a turbidity of 1.0 and seeded on IsoSensitest agar (Oxoid). Etest strips (Biodisk AB) were placed on top followed by anaerobic incubation at 37°C for 48 h. PCR ribotyping of *C. difficile* isolates. Preparation of template, PCR reaction, separation of PCR products and analysis of banding patterns were according to Stubbs et al (1999, J. Clin. Microbiol. 37:461–463) with minor modifications (Norén et al, 2004, J. Clin. Microbiol. 42:3635–3643) and, accordingly, use of our own nomenclature (prefix SE, Sweden).
Results: In 2004 4% of the clinical isolates showed moxifloxacin resistance (MIC > 32 mg/l) and in 2007 we noted a significant increase of resistant isolates, i.e. the prevalence of resistance was almost six times as high (22.5%). The antibiograms indicated epidemic SE17 uniformly and current nosocomial clusters of disease were located mainly in two geographically distant Dialysis departments and in the Department of Infectious diseases.
Conclusion: Moxafloxacin resistance may not only be a selecting factor and screening marker for NAP1/027 but also useful to detect other virulent *C. difficile* like for example SE17 (Ribotype 012) in Sweden.

P1210 Antibiotic susceptibility pattern and tetracycline resistance of *Lactobacillus* strains of different origin

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During the last years substantial attention has been paid to the fact that resistance determinants are widespread among isolates from non-clinical settings. It has been proposed that some food starter cultures and probiotic additives might contain antibiotic resistance genes, particularly to the widely used antibiotic tetracycline.
The aim of the study was to test the possibilities for safety assessment of *Lactobacillus* strains from food by comparing their phenotypic and genotypic antibiotic resistance with reference, intestinal and agricultural strains.
Methods: A total of 44 strains of *L. paracasei* and *L. plantarum* were tested: 25 human (13/12), 4 reference (2/2), 13 silage (7/6) and 2 food industrial (1/1) strains. The MIC values of lactobacilli to ampicillin (AM), gentamicin (GM), streptomycin (SM), erythromycin (EM), tetracycline (TC), chloramphenicol (CL) were assessed according to EU Commission recommendations. Microbial DNA was extracted using QIAamp DNA Kit. Integrase Int1 and tetracycline resistance genes tet(M), (S), (O), (K), (L) were detected by PCR method.
Results: *L. paracasei* and *L. plantarum* strains were susceptible to all tested antibiotics. Compared with *L. paracasei L. plantarum* strains showed higher MIC values to TC($p < 0.001$), EM($p = 0.014$), CL($p = 0.011$) while to AM($p = 0.01$), GM($p < 0.001$) and SM($p = 0.001$) the in former the MIC values were higher than in latter. No differences in MIC values were seen between the food and intestinal & agricultural strains.
All *L. paracasei* and 19/21 *L. plantarum* strains contained IntI gene. The tet genes (M, K, L) alone in different combination were present in 14/23 *L. paracasei* and 16/21 *L. plantarum* strains, respectively. The food *L. paracasei* strain did not contain any tet genes and expressed the low TC resistance (0.19 μg/ml). Tet(K) gene was more frequently detected in *L. plantarum* (16/21 vs 4/23;$p < 0.001$) strains and more widely distributed among human isolates as compared to silage & food strains (15/25 vs. 3/15;$p = 0.012$). The food *L. plantarum* strain did not possess the tet(K) gene but tet(L) and (M) were found resulting in moderate TC resistance (6 μg/ml).
Conclusion: *Lactobacillus* strains of different origin without phenotypical resistance may contain genetic determinants, e.g. integrons and tet-res genes. The comparison of the *Lactobacillus* food strains with reference, intestinal and agricultural strains by phenotypic and genotypic antibiotic resistance methods enables to assess their safety for commercial use.

ESBLs and other β-lactamases – Part 2

P1211 Plasmid-encoded and class 3 integron-associated extended-spectrum β-lactamase gene blaGES-1 from *Escherichia coli* in Switzerland

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Objectives: *Escherichia coli* isolate TB7 showing an extended-spectrum β-lactamase (ESBL) phenotype was isolated in 1998 from urine of a Swiss patient who had no recent travel history. That strain was resistant to most β-lactams, except piperacillin-tazobactam, cephamycins and carbapenems, with a synergy image between clavulanic acid and extended-spectrum cephalosporins. In addition, a peculiar synergy between cefoxitin and cefuroxime was noticed. The aim of the present study was to identify the ESBL gene and the genetic structures at the origin of its acquisition.
Methods: Genomic DNA *E. coli* TB7 was extracted and used for PCR screening of known ESBL genes. Plasmids were extracted using the

Kieser technique. Mating-out assays were performed using *E. coli* J53 as recipient strain and a selection with ceftazidime (30 mg/L)- and azide (100 mg/L)-containing media. Class 1 and class 3 integron features were mapped by PCR using specific primers.
Results: PCR and sequencing showed that *E. coli* TB7 expressed the ESBL GES-1. Mating-out assays remained unsuccessful but electro-transformation allowed to obtain transformant *E. coli* (pTB7) harbouring the blaGES-1 gene on a 12-kb plasmid. *E. coli* (pTB7) expressed an ESBL phenotype consistent with GES-1 expression, and was resistant in addition to amikacin and kanamycin, but susceptible to all other compounds, including sulfonamides. PCR mapping did not allow identification of class 1 integron features whereas blaGES-1 is usually associated to those structures. Further screening evidenced class 3 integron features, with the Int3 integrase encoding gene located upstream of blaGES-1. Downstream of blaGES-1, a repC gene encoding a putative replicase related to those of broad-host range IncQ plasmids was found.
Conclusion: This study identified ESBL GES-1 in Switzerland for the first time. This is one of the very few reports of acquisition of antibiotic resistance gene mediated by a class 3 integron. Noteworthy, the same blaGES-1-class 3 integron structure association had been evidenced onto a plasmid from a *Klebsiella pneumoniae* isolate recovered in 1999 in Portugal. This finding strengthens the dissemination of GES-1 enzyme worldwide, after its identification in France, Portugal, Argentina and Brazil.

P1212 First isolation of GES extended-spectrum β-lactamases in Enterobactericeae in Belgium

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Objectives: Extended-spectrum β-lactamase (ESBL) producing Enterobactericeae strains are increasing worldwide, mainly due to the emergence of community- and hospital-acquired CTX-M enzymes. ESBLs of the GES-type (also named IBC) are reported in several Gram-negative rods, including *P. aeruginosa*, *E. coli*, and *K. pneumoniae* isolates from Greece, South Africa and Asia. GES-type ESBLs have the property to extend their spectrum of activity against carbapenems by single point mutations. Here, we describe the emergence of GES-6-producing *Citrobacter freundii* and *Enterobacter cloacae* strains in Belgium.
Methods: Clinical isolates were identified using standard microbiological techniques and resistance profile was determined by disc diffusion methods. ESBL genes were sought by PCR and genetic structures surrounding the blaGES-type gene were characterised by cloning, PCR and sequencing. The strains were analysed by plasmid extraction, conjugation and electroporation assays.
Results: In May 2007, a single *C. freundii* and a single *E. cloacae* isolate were recovered respectively from urine and stool specimens of two children hospitalised together in the same paediatric ward at UCL-Saint-Luc hospital, Brussels. The index patient, a 2-year old girl with a diagnosis of congenital stenosis of the biliary tract had been transferred from Israel to Belgium for liver transplantation surgery. Systematic stool screening upon hospital admission revealed the presence of an ESBL-producing *E. cloacae* in this patient. The two strains had a similar resistance pattern, (ceftazidime, amikacin, cotrimoxazole). A synergy was observed by phenotypic confirmatory tests using ceftazidime and ceftazidime/clavulanate double discs. PCR-sequencing revealed the presence of blaGES-6 in both isolates. blaGES-6 was integron-located together with 4 other gene cassettes (aacA4, smr, dhfrI and aadA1). The blaGES gene was inserted onto a conjugative plasmid of 120-kb present in both strains, suggesting in vivo horizontal transfer. The transconjugants displayed an ESBL phenotype but spares C4 cephalosporinases and carbapenems.
Conclusion: This work illustrates the importance of international transfer in the spread of GES-type-producing Enterobacterial strains and suggests that selection of point mutant derivatives of GES-type enzymes, extending their spectrum towards carbapenem is to fear in the next future.

P1213 Molecular characterisation of PER-1 extended-spectrum β-lactamase producing *Pseudomonas aeruginosa* clinical isolates from Romania

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Objectives: The presence of PER-1-producing *P. aeruginosa* isolates in Romania has been already described since 2005. The aim of the present study was to characterise the genetic structures associated with the bla genes harboured by these strains and to establish any clonal relationships between the isolates.
Methods: Analytical isoelectric focusing was used for preliminary characterisation of the β-lactamases expressed by the isolates included in this study. PCR using blaPER-1, blaOXA-2, and blaOXA-10 specific primers, followed by DNA sequencing, was performed in order to identify β-lactamase genes present in *P. aeruginosa* isolates. The occurrence of class1 integrons and Tn1213 transposon was assessed by PCR. Amplicons were further analysed by RFLP (restriction fragment length polymorphism) technique using several combinations of restriction endonucleases. Clonal relationships of PER-1 producing isolates were established by pulsed-field gel electrophoresis (PFGE) analysis of macrorestriction patterns of genomic DNA.
Results: Seventy-four out of 94 ceftazidime resistant clinical isolates examined were PCR-positive for blaPER-1. In all of the studied isolates the blaPER-1 gene was located within a composite transposon Tn1213, bracketed by two different insertion sequences, ISPa12 and ISPa13 (Poirel et al, 2005, Antimicrob. Agents Chemother.: 1708–1713). Additionally, PCR experiments revealed the presence of a pair of class 1 integrons of 3350 bp and 3900 bp among the majority of isolates. The 3350 bp class 1 integron harboured blaOXA-74 gene in the first gene cassette position and the aminoglycoside resistance gene aacA4 in the second place. The blaOXA-2 gene is located within the variable region of the 3900bp integron. The PER-1 producing isolates, originating from nine Bucharest hospitals, clustered into four PFGE types with a high degree of similarity.
Conclusions: PER-1 appears to have a significant presence among the clinical isolates from Bucharest hospitals. PER-1 expression was inferred to be the major source of oxyimino-β-lactam resistance in the examined isolates although they also produced other β-lactamases. Along with some recent reports from other European countries, this work illustrates the ongoing dissemination of organisms with PER-type extended-spectrum β-lactamase.

P1214 Molecular diversity of VEB-1 extended-spectrum β-lactamase-producing *Pseudomonas aeruginosa* in Bulgarian hospitals

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Objectives: *Pseudomonas aeruginosa* causes 10–15% of the nosocomial infections in the world and in the recent years it constitutes for more than 35% of the hospital acquired infections in Bulgaria. The emergence of a large number of VEB-1 ESBL-producing strains raised the need for their molecular characterisation.
Methods: During the years 2000–2007, we examined a total of 111 ceftazidime resistant *P. aeruginosa* from more than 25 hospitals across the country. Identification of the strains and their antibiotic susceptibility were determined by Mini API (BioMerieux, France) and the disc-diffusion method. Specific PCR was carried out for detection of the blaVEB-1 gene. A high resolution multilocus variable number of tandem repeat analysis (MLVA6) modified from a previously reported scheme (Onteniente et al.) was applied for studying the molecular diversity among the isolates.
Results: The presence of the chromosomal gene blaVEB-1 was detected in 73% (81/111) of the isolates. The modified MLVA6 scheme proved highly discriminatory generating a total of 18 genotypes. More than 35% of the isolates were grouped within a single cluster that was prevalent

in the larger city and university hospitals. The other 17 genotypes were presented with a smaller number of isolates (up to six) and often with local distribution.

Conclusions: VEB-1 ESBL-producing *P. aeruginosa* are widespread in Asia but in Europe are reported only in France. Several recent studies highlight their importance among the Bulgarian hospitals and this is the first one to investigate the molecular diversity within a large and representative strain collection. The modified MLVA6 typing scheme accurately discriminated both closely related and unrelated isolates from the same hospital. The provided data suggest that a single cluster is largely distributed among the Bulgarian hospitals.

P1215 Characterisation of an outbreak of nosocomial infection caused by a multiresistant *Acinetobacter baumannii* producing OXA-30 type-β-lactamase

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Objectives: 1. Analysis of the molecular mechanisms involved in the β-lactam resistance of multidrug-resistant *A. baumannii* isolates; 2. the phenotypically and genotypically comparison of clinical and environmental outbreak strains to those obtained before the outbreak.

Methods and Results: Forty multiresistant-drug *A. baumannii* (MDRAB) isolates were consisted of: 8 preoutbreak isolates, 27 clinical and 5 environmental outbreak isolates. Thirty-five clinical isolates were recovered from twenty-six patients admitted to different wards belonging to Amiens University Hospital (AUH), France. MDRAB outbreak strains were observed in the orthopaedic and plastic surgery wards.

The *A. baumannii* isolates were resistant to all of β-lactams tested (MICs, 48–>256 ug/mL) except imipenem (MIC < 4 ug/mL), to aminoglycosides (MICs, 24–>256 ug/mL), and to ciprofloxacin (MIC > 256 ug/mL). All isolates remained susceptible to colistin (MIC < 2 ug/mL).

The frequency of intl1 gene carriage was 96.8% and 37.8% in the outbreak and preoutbreak isolates respectively.

By using isoelectric focusing, three β-lactamases were detected: one produced a band with a pI > 8.0, probably corresponding to a chromosomal AmpC cephalosporinase, the second focused at pI 5.4 and was consistent to TEM-1, the third focused at pI 7.3 and corresponded to OXA-30 type.

blaOXA-30 analysis revealed 3 gene cassettes: intl1 gene with 459-bp coding for an integrase; aacA4 gene with 759-bp encoding a 6′-N-aminoglycoside acetyl transferase and blaOXA-30 gene with 852-bp coding for OXA-30 β-lactamase, with ATG as initiation codon and TAA as termination codon. The deduced amino acid sequence showed an enzyme of 276 amino acids long with Ser-Thr-Phe-Lys at positions 71 to 74, Tyr-Gly-Asn at positions 144 to 146, and Lys-Thr-Gly at positions 215 to 217. blaOXA-30 differed from blaOXA-1 by one base at codon (AGA 131-GGA) resulting in amino acid substitution Arg128-Gly. Pulsed Field Gel Electrophoresis with ApaI showed a single pattern (A) in outbreak and environmental isolates, and 6 unrelated genotypes (B-G) in preoutbreak isolates.

Conclusion: The present work is the first report of *A. baumannii* clinical isolates in France that produce an OXA-30 β-lactamase.

P1216 The first detection of OXA-35 β-lactamase in *Pseudomonas aeruginosa* clinical isolates in Poland and identification of OXA-118, a new extended-spectrum variant of OXA-10 β-lactamase

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Objectives: The OXA-type enzymes are frequently observed in clinical isolates of *P. aeruginosa*. Most of the OXA-type ESBLs are OXA-10 derivatives. Four of these ESBLs (OXA-13, OXA-13–1, OXA-19 and OXA-28) and OXA-35 (restricted-spectrum β-lactamase) differ in one or two amino acids substitutions. Moreover, the structures of class 1 integrons carrying blaOXA-13, blaOXA-13–1, blaOXA-19, blaOXA-28 and blaOXA-35 genes are very similar. Probably OXA-35 is the progenitor of OXA-13-like ESBLs.

The aim of the study was to identify strains producing OXA-10-like ESBLs, among *P. aeruginosa* isolated in two Warsaw's hospitals.

Methods: *P. aeruginosa* strains (n = 30) were isolated from various clinical specimens obtained from patients in A and B Warsaw's hospitals. The presence of ESBLs was detected by a double-discs synergy test. MICs values of antibiotics were determined by agar dilution methods, according to CLSI recommendation. The presence of class 1 integron and genes coding OXA-10-like enzymes was detected by PCR. Gene cassettes were identified by sequencing the obtained amplicons.

Results: The production of ESBLs by 40 *P. aeruginosa* strains was expected in case of resistance to broad-spectrum cephalosporins or aztreonam. Seven of these isolates were found to contain OXA-10-like enzymes located in a class 1 integrons. In 6 *P. aeruginosa* isolates from hospital A the identical structure of integron was identified. This integron contained aac(6′)-Ib and blaOXA-35 genes. The 6 isolates carrying this integron were resistant to cefepime but 5 of them were also resistant to aztreonam and cefotaxime. Among strains from hospital B only in one *P. aeruginosa* isolate, gene coding OXA-10-like enzyme was detected. This gene encoded the new extended-spectrum variant of OXA-10 β-lactamase (named OXA-118). Sequencing showed that OXA-118 was identical to OXA-10 except two amino acids. The clinical isolate producing OXA-118 exhibited the inhibitor-sensitive phenotype (between imipenem and ceftazidime as well as cefepime).

Conclusion: OXA-35 β-lactamase producing *P. aeruginosa* strains were identified for the first time in Poland. The presence of blaOXA-35 gene, within integron in 6 clinical isolates suggests the possibility to create new OXA-13-like ESBLs in Polish hospitals. Moreover, the new extended-spectrum β-lactamase OXA-118, variant of OXA-10, encoded by integron-located gene was identified in *P. aeruginosa* clinical isolate.

P1217 Characterisation of a naturally-occurring oxacillinase from *Achromobacter xylosoxidans*

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Objectives: *Achromobacter xylosoxidans* (previously *Alcaligenes xylosoxidans*) is a Gram-negative, glucose-non-fermenting organism that is increasingly recognised as a nosocomial pathogen. It is reported as variably resistant to β-lactam antibiotics. The aim of the present study was to identify the naturally-occurring β-lactamase gene of *A. xylosoxidans*.

Methods: *A. xylosoxidans* CIP69598 was used for cloning. Genomic DNA was partially digested with Sau3AI and ligated into pBK-CMV followed by electro-transformation in *E. coli* TOP10. Selection of recombinant *E. coli* strains was made on ticarcillin and kanamycin-containing plates. Detection of bla(OXA-114a)-like genes in clinical strains of *A. xylosoxidans* was performed by PCR. Purification of OXA-114a was performed by ion-exchange chromatography. Kinetic parameters of OXA-114a were determined by UV spectrophotometry. Inducibility of β-lactamase production was tested using imipenem and cefoxitin as inducers

Results: A recombinant strain *E. coli* TOP10 (p6S3) was obtained that was resistant to amoxicillin and piperacillin, a phenotype not antagonised by clavulanic acid or tazobactam addition. Plasmid p6S3 possessed a 1,176-bp insert comprising a novel oxacillinase gene encoding OXA-114a. Conserved motifs of serine β-lactamase as well as those specific of oxacillinases were identified. The highest amino acid identity (56%) was observed with a putative oxacillinase of *Burkholderia cepacia*. Kinetic parameters indicated robust hydrolysis of piperacillin and cephalothin, and to a lesser extent, ticarcillin. No hydrolysis of oxacillin or third-generation cephalosporins was observed. Hydrolysis of imipenem was noticed, but did not correlate with the susceptibility of the source strain. Four other OXA-114-like sequences (OXA-114b to OXA-114e) with no more than four amino acids difference between each other, were

identified from the four clinical strains. No induction of OXA-114-like enzymes was detected in clinical isolates.

Conclusion: This study identified a naturally-occurring β-lactamase of *A. xylosoxidans*. It is likely that the contribution of OXA-114-like enzymes in the final β-lactam resistance profile of most *A. xylosoxidans* strains may be secondary given its rather narrow spectrum of activity. This description further strengthens that numerous Gram-negative aerobes harbour oxacillinase genes in their chromosome.

P1218 Spread of diverse AmpC-type-β-lactamase-producing *E. coli* in Madrid, Spain: epidemiology and molecular characterisation

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Objectives: Little is known about *E. coli* non-susceptible to 3rd generation cephalosporins but non ESBL-non-producers. The resistance mechanisms and the molecular epidemiology of *E. coli* isolates with antibiotic resistance profile compatible with AmpC-mediated resistance were studied.

Methods: From January-2006 to December-2007 2,349 *E. coli* isolates (only one isolate by patient) non-susceptible to 3rd generation cephalosporins were collected by 2 hospitals and 1 community healthcare centre in Madrid, Spain. Antimicrobial susceptibility testing and ESBL detection was performed following CLSI guidelines. All isolates resistant to cefoxitin, amoxicillin–clavulanic acid, and the 3rd generation cephalosporins with no clavulanic acid synergistic effect were subjected to: a) molecular characterisation of resistance mechanisms by PCR and sequenciation; b) Molecular epidemiology by PFGE after XbaI digestion.

Results: A total of 143 (6.1%) isolates accomplishing the selection criteria; 48 (33.5%) were plasmid mediated AmpC (pAmpC) (45 CMY-2, 2 DHA-1, 1 CMY-4) and 112 (78.3%) were chromosomal AmpC hyperproducers (hAmpC). Seventeen isolates (11.9%) had both mechanisms of resistance pAmpC and hAmpC. In addition, 15 isolates (2 pAmpC and 13 hAmpC) were also ESBL producers, 10 CTX-M-15, 3 CTX-M-14 and 2 CTX-M-9. Mutations in the promoter of chromosomal ampC gene included 2 main profiles: a) –88, –82, –42, –18, –1, +58; detected in 77 (68.7%) isolates; and b) at least 3 of the following: –73, –32, –28, +82; detected in 22 (19.6%). By PFGE, only small clusters were detected affecting mainly CMY-2 and CTX-M-15 producers.

Conclusions: Emergence of the pAmpC CMY-2 and hAmpC in *E. coli* is a relevant problem. In addition, the presence of AmpC production in an epidemic *E. coli* CTX-M-15 producer suggests that the association of both mechanisms of resistance may become an important public health issue.

P1219 Plasmid-mediated CMY-2 AmpC β-lactamase and CTX-M-14 extended-spectrum β-lactamase in *Proteus mirabilis* clinical isolate

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Objectives: *Proteus mirabilis* is considered one of the most β-lactam susceptible members of the Enterobacteriaceae. Although extended-spectrum β-lactamases (ESBLs) have recently been identified, there are few reports of plasmid-mediated AmpC β-lactamase (PCBL) in *P. mirabilis*. In general, ESBLs confer resistance to oxyimino-cephalosporins but not cephamycins and are inhibited by β-lactamase inhibitors, while PCBLs provide resistance to cephamycins and oxyimino-cephalosporins and are refractory to β-lactamase inhibitors. We report the identification of ESBL and PCBL enzymes in *P. mirabilis* isolate.

Methods: *P. mirabilis* 10830 isolated from neurological abscess was collected in Hospital de Santa Maria at Lisboa. Susceptibility to antimicrobial agents was determined by disk diffusion test, following CLSI guidelines. The conjugation assay was performed using *Escherichia coli* 802 K as recipient strain. PCR experiments were performed using specific primers for ESBL, PCBL, insertion sequences and class 1 integrons. Plasmid extraction was prepared using Kado & Liu method.

Results: The isolate showed resistance to amoxicillin-clavulanate, cefoxitin, cefpime, cefotaxime; intermediate to ceftazidime and susceptible to imipenem and fluoroquinolones. It was observed two plasmids in both *P. mirabilis* and transconjugant *E. coli* P10830, which showed the same susceptibility to β-lactams including cefoxitin.

The double-disk synergy test was observed between clavulanate and cephalosporins (cefepime and cefotaxime). However, a higher synergic effect was observed when the cloxacillin was applied to Mueller-Hinton agar. PCR and sequencing revealed that *P. mirabilis* 10830 clinical isolate and the transconjugant *E. coli* K P10830 harboured CMY-2 AmpC β-lactamase and CTX-M-14 extended-spectrum β-lactamase; A complex class 1 integron containing the blaCMY-2 gene; and the blaCTX-M-14 gene was bracketed upstream and downstream by insertion sequences ISEcp1 and IS903, respectively.

Conclusions: This is the first report of a plasmid that carries both CTX-M-type ESBL and CMY-type PCBL genes. The finding of the coexistence of these genes in such a transmissible plasmid that can propagate in different hosts provides further insight into the mechanisms of transmission of these β-lactamases in Portugal.

P1220 Expression of blaCMY-14 promoted by insertion sequence ISEcp1 in *P. mirabilis* isolates in Greece

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Objectives: *Proteus mirabilis* clinical strains with β-lactam resistance phenotype consistent with production of an AmpC-type β-lactamase are isolated with increased frequency in our hospital. The aim of this study was to identify the mechanism of the high-level expression of blaCMY-14 and blaCMY-2 genes which are responsible for this phenotype.

Methods: Sixteen nonreplicate *P. mirabilis* clinical isolates were analysed. Identification and susceptibility testing were carried out following standard procedures. Molecular typing was performed with REP-PCR. β-lactamase production was analysed by isoelectric focusing (IEF). β-lactamase genes were determined by PCR and sequencing. The ISEcp1 element was detected in the 5′-end of blaCMY genes by PCR. Sequencing of the resulting amplicons was performed with the same primers. Sequence similarity searches were carried out with the BLAST program found at the website of the NCBI.

Results: All isolates were resistant to ampicillin, amoxicillin-clavulanate, cefotaxime, ceftazidime, cefoxitin and fluoroquinolones; susceptible to cefepime, aztreonam, piperacillin/tazobactam and carbapenems. Isolates were obtained during the period Oct 2005 to Feb 2007 from urine (3), pus (5), sputum (4) or faeces (colonisers) (4) from inpatients in ICU (7), Internal Medicine department (4) and Surgery department (5). IEF analysis revealed the presence of β-lactamases of pI 5.4 and >8.4. PCR analysis revealed the presence of CMY- and TEM-type genes. Sequencing showed the presence of CMY-14 gene in 14 isolates and of CMY-2 in two isolates. Extensive analysis showed that ISEcp1 was responsible for mobilisation of a transposition unit including itself and blaCMY-14 or blaCMY-2. All isolates carried the ISEcp1 mobile element at the same distance upstream of the blaCMY gene and in four representative isolates the sequences between them were identical. REP-PCR showed that isolates were grouped in two main clones probably related to each other.

Conclusions: CMY-14 reported in Poland was found in *P. mirabilis* isolates resistant to cefotaxime, ceftazidime and cefoxitin in Greece. The putative promoter element of ISEcp1 is necessary for the high-level expression of blaCMY-14 to confer resistance to extended-spectrum cephalosporins. The present study underscores the spread and evolution of the AmpC-producing *P. mirabilis* in our country.

P1221 Prevalence of plasmid mediated AmpC β-lactamases in non-*typhi* salmonella from Ireland

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Objectives: The genes encoding AmpC β-lactamases occur naturally on the chromosome of several members of the Enterobactericeae with

the exception of *Klebsiella* spp., *Salmonella* spp. and *Proteus mirabilis*. Since the 1980s the occurrence of plasmid mediated AmpC β-lactamases has been reported with increasing frequency. The aim of this study was to determine the presence of plasmid mediated AmpC β-lactamases in non-Typhi *Salmonella* from Ireland.

Methods: The National Salmonella Reference Laboratory (NSRL) of Ireland screens isolates received for susceptibility to a panel of antimicrobial agents including ampicillin, ceftazidime, cefotaxime, and cefpodoxime, in accordance with CLSI disk diffusion methods. Isolates with reduced susceptibility to the expanded spectrum cephalosporins are further screened for susceptibility to cefoxitin and meropenem and ESBL production is confirmed by the CLSI combination disk method using cefpodoxime and cefpodoxime/clavulanate. Isolates resistant to cefoxitin and cefpodoxime/clavulanate were considered suspect AmpC β-lactamase producers. Antimicrobial susceptibility test data for 8216 isolates received between January 1st 2000 and October 31st 2007 was reviewed for suspect AmpC β-lactamase production. Screen positive isolates were examined for the presence of genes encoding 6 phylogenetic groups of plasmid-mediated AmpC β-lactamases using a multiplex PCR assay (Pérez-Pérez & Hanson. J Clin Microbiol 2002, 40: 2153–62). The full coding region of the genes identified were sequenced using specific primers.

Results: Seven isolates were identified as suspect AmpC producers by the screen criteria applied. PCR and sequencing of the full genes confirmed the presence of blaCMY-2 in 3 isolates (2 human isolates of S. Typhimurium isolated in 2007 and 1 human isolate of S. Virchow isolated in 2006) and blaACC-1 in a single isolate of S. Bareilly isolated from seawater in 2000.

Conclusions: This is the first report of plasmid mediated AmpC β-lactamases in non-Typhi *Salmonella* from Ireland.

P1222 Expression analysis of the native ampC gene in Norwegian clinical isolates of *Escherichia coli* with defined ampC promoter mutations and an AmpC-resistance profile

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Objective: The objective was to examine native ampC mRNA levels by quantitative RT-PCR (qRT-PCR) in Norwegian clinical isolates of *Escherichia coli* with defined ampC promoter-attenuator mutations and relate those to minimum inhibition concentrations (MICs) of cefoxitin, third generation cephalosporins, piperacillin-tazobactam, and aztreonam.

Methods: The Norwegian Reference Centre for Detection of Antimicrobial Resistance (K-res) received 106 clinical isolates of *E. coli* from different Norwegian laboratories with reduced susceptibility to third-generation cephalosporins without clavulanic acid synergy, from 2003 to 2005. Of these, twelve were non-plasmid mediated blaampC strains, with defined ampC promoter and attenuator alterations compared to the *E. coli* wild-type ampC promoter-attenuator. Expression of the native ampC gene was examined by qRT-PCR. ampC mRNA levels, normalised to the expression of the reference gene gapA, were compared to those in six cefoxitin-susceptible isolates of *E. coli*, including ATCC 25922. MICs were determined by Etest.

Results: The 12 isolates showed a 27 to 380-fold increase in ampC mRNA levels compared to the ampC mRNA level in *E. coli* ATCC 25922. The ampC mRNA levels varied within the different promoter-attenuator mutation groups. A single strain had an ISEc10 element inserted between the –35 and –10 boxes, providing an almost perfect *E. coli* –35 box 17 bp upstream of the endogenous –10 box. This isolate showed a 54-fold increase in ampC mRNA level. The ampC mRNA levels in the five cefoxitin-susceptible clinical isolates of *E. coli* varied from 0.7 to 2.2 fold, suggesting that the mutations observed in these isolates have no or minor effect on transcription of the ampC gene. For the isolates with reduced susceptibility to third-generation cephalosporins we did not observe any significant correlation between ampC mRNA levels and MICs of FOX, CPD, CTX, CAZ, TZP, and ATM.

Conclusions: (i) Clinical isolates of *E. coli* with defined ampC promoter-attenuator alterations overexpressed ampC 27–380-fold compared to *E. coli* ATCC 25922. (ii) We did not find any significant correlation between ampC mRNA levels and MICs of cefoxitin, third generation cephalosporins, aztreonam and piperacillin-tazobactam.

P1223 Wide occurrence of plasmid-mediated AmpC β-lactamase-producing *Escherichia coli*, *Klebsiella* spp., *Proteus mirabilis*, and *Salmonella* spp. isolates in Korea: results of a multicentre survey in 2007

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Objectives: Plasmid-mediated AmpC β-lactamases (PABLs) are cephalosporinases that confer resistance to a wide variety of β-lactam drugs and that may thereby create serious therapeutic problems. The PABL-producing organisms are a major concern in nosocomial infections and should therefore be monitored in surveillance studies. The present study was conducted to determine the epidemiology and genotypic distributions of PABL-producing isolates in Korea.

Methods: During the period of May to August 2007, a total of 1,350 consecutive and nonrepeat isolates of *Escherichia coli*, *Klebsiella* spp., *Proteus mirabilis*, and *Salmonella* spp. were collected at 12 hospitals in Korea. The 248 (18.4%) cefoxitin non-susceptible isolates tested for multiplex AmpC PCR and DNA sequencing. The PABL-positive isolates were further investigated for antimicrobial susceptibility by broth microdilution methodology and extended-spectrum β-lactamases (ESBLs) by boronic acid disk tests.

Results: PABL producers were found at all the 12 sites in Korea. PABLs were found in 15/577 *E. coli* (2.6%; 9 of DHA-like, 5 of CMY-like, and 1 of ACT-1-like); 152/544 *K. pneumoniae* (27.9%; 149 of DHA-like, 1 of CMY-like, and 2 of ACT-1-like); 6/65 *K. oxytoca* (9.2%; 6 of DHA-like); 3/104 *P. mirabilis* (2.9%; 1 of DHA-like and 2 of CMY-like); and 1/60 *Salmonella* spp. (1.7%; 1 of CMY-like) isolates. One hundred and four of the 152 PABL-producing *K. pneumoniae* isolates (68.4%) coproduced ESBLs. Susceptibilities of the PABL-producing *K. pneumoniae* were as follows: ceftazidime, 3%; cefotaxime, 26%; aztreonam, 26%; cefepime, 79%; imipenem, 99%; gentamicin, 12%; amikacin, 14%; and ciprofloxacin, 11%.

Conclusion: PABL producers were found to be widespread in Korea. A DHA type is the most prevalent enzyme and the DHA-producing *K. pneumoniae* isolates have rapidly increased in Korea. Each of the PABLs is associated with multidrug resistance. Clinical laboratories should be able to detect PABLs to ensure optimal patient care and infection control.

P1224 The association of several amino acid substitutions in the extended-spectrum AmpC β-lactamases from *Escherichia coli* decreases its overall enzymatic activity

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Objectives: class C β-lactamases (AmpC) weakly hydrolyse extended-spectrum cephalosporins (ESCs) and carbapenems. Recently, a novel mechanism of resistance, the extended-spectrum AmpC β-lactamases (ESACs), have emerged among enterobacterial clinical isolates. These variants exhibited increased catalytic efficiencies toward ESCs, leading to enhanced MICs of ESCs. The structural modifications responsible for the broadened hydrolysis-spectrum, mainly amino acid substitutions, occurred in secondary structures surrounding the binding site: the helix H-9, the helix H-10, the helix H-11, the R2 loop, and the Omega loop. The aim of this study was to evaluate if the combination of several amino acid replacements in ESACs could further enlarge the hydrolysis spectrum of these β-lactamases.

Methods: Site directed mutagenesis was applied to the ampC EC2 gene that encoded a wild-type cephalosporinase of *E. coli*. The resistance patterns of variants presenting a single substitution at position 219

(Omega loop), 246 (helix H-11), 287 (helix H-9), and 298 (R2 loop) were compared to those of four variants with the following double substitutions: E219K+S287N; S287N+N346I; S287N+E219K; and V298L+N346I.

Results: The single variants S287N and V298L conferred the highest level of resistance towards ESCs, such as ceftazidime and cefepime, whereas the MICs for the single variants E219K and N346I were only slightly modified. All the double variants displayed decreased MICs of ESCs as compared to those of the single variants S287N or V298L. In addition, the MICs of imipenem remained unchanged for all variants as compared to the wild-type *E. coli* reference strain.

Conclusion: Double substitution at critical positions in AmpC β-lactamase of *E. coli* do not provide enhanced spectrum of hydrolysis.

P1225 Presence and epidemiology of the IRT β-lactamases in contemporary *Escherichia coli* isolates in a hospital in Madrid, Spain

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Objectives: Recent data of inhibitor resistant TEM (IRT) β-lactamases are scarce. We determined the presence and epidemiology of IRT β-lactamases in contemporary *E. coli* isolates with reduced susceptibility to amoxicillin/clavulanate (AMC) recovered in our hospital.

Methods: *E. coli* isolates with reduced susceptibility to AMC (MIC ⩾16/8 mg/L) (WIDER System, Fco. Soria Melguizo, Spain) were prospectively collected in our hospital between September-2006 and March-2007. Two additional susceptibility tests, MIC-agar dilution (AMC fixed 2:1 ratio and 4 mg/L-fixed clavulanate concentration) and disc-diffusion were performed. β-lactam susceptibility phenotypes were further analysed and IRTs were characterised by isoelectric focusing, PCR and sequencing. Their population structure (XbaI-PFGE) was also determined.

Results: 152 out of 3,556 (4.3%) *E. coli* isolates (40.2% recovered from hospitalised and 59.8% from outpatients) had reduced susceptibility to AMC (36.2% resistant and 63.8% intermediate, CLSI-2007). Sources of isolation were: urine (73.7%), blood (9.8%), wounds (5.3%), respiratory secretions (4.6%), and others (6.6%). Analysis of the β-lactam-resistance profiles of 99 randomly selected isolates revealed that reduced susceptibility to AMC could be ascribed to penicillinase (TEM-1) hyperproduction (60.6%), extended-spectrum β-lactamase synthesis (15.2%), IRT synthesis (12.1%) or constitutive AmpC overproduction (12.1%). Confirmed IRT-producing *E. coli* isolates (n = 32) were mostly from urine (66.6%). Five (41.7%) isolates were from hospitalised and 7 (58.3%) from outpatients. No significant discrepancies were obtained between the different susceptibility testing methods, although IRT phenotype was better detected with 4 mg/L fixed clavulanate concentration. Molecular characterisation of bla genes revealed that the IRTs were: 4, TEM-30/IRT-2 (pI 5.2); 2, TEM-32/IRT-3 (pI 5.4); 2, TEM-37/IRT-8 (pI 5.2); 1 TEM-33/IRT-5 (pI 5.4); 1 TEM-36/IRT-7 (pI 5.2); 1 TEM-34/IRT-6 (pI 5.4), and 1, TEM-54 (pI 5.4). IRT-*E. coli* had unrelated PFGE patterns.

Conclusion: The IRT-producing *E. coli* isolates recovered in our hospital over a 7-month period, most from outpatients with urinary-tract infections, represent 12.1% of isolates exhibiting reduced susceptibility to AMC. Interestingly, 7 different IRTs were present in non-related isolates suggesting an independent emergence of these TEM variants mainly attributable to antibiotic selective pressure.

P1226 Molecular epidemiology and mechanisms of β-lactam resistance in *Klebsiella pneumoniae* isolates with reduced susceptibility to amoxicillin-clavulanate from a chronic-care hospital

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Objective: A high rate of decreased susceptibility to amoxicillin-clavulanate (A/C) was noticed amid *K. pneumoniae* isolates from a chronic-care hospital. The aim of this study was to investigate their molecular epidemiology and the mechanisms involved in β-lactam resistance.

Methods: 94 nonrepetitive *K. pneumoniae* isolates recovered between January 2006 and November 2007 from patients admitted to the above institution were included in the study. Susceptibility testing was achieved by microdilution or disk diffusion. The presence of integrons and of oxa-1, tem-1 like, shv-1 like, pse-1 (β-lactam resistance) and aac(6′)-1b (aminoglycoside resistance) genes was investigated by PCR. Epidemiological relatedness among isolates with reduced susceptibility to A/C was studied by REP-PCR.

Results: (a) 46.7% (29/62) and 18.8% (6/32) of isolates collected during 2006 and 2007 were intermediate-resistant to A/C – MIC 16 mg/L. (b) 94.2% (33/35) of these isolates showed a phenotype consistent with production of inhibitor-resistant TEM (IRT) or OXA β-lactamases, since they were fully susceptible to cefazolin; the presence of tem-1 like plus shv-1-like and of oxa-1 plus shv-1-like was demonstrated in 45.5% and 54.5% of them. (c) All oxa-1-positive isolates were additionally resistant to nitrofurantoin, tobramycin (Tob), kanamycin (K), nalidixic acid, ciprofloxacin, chloramphenicol and sulphonamides and harboured aac(6′)-1b, which confers resistance to Tob and K. No integrons could be detected – except for a single isolate with an integron of 0.8 Kb – with 5′CS and 3′CS primers, although amplicons of ca. 0.7 and 1.8 Kb were obtained when using 5′CS together with aac(6′)-1b or oxa-1 specific reverse primers. (d) Four different REP patterns were identified in the 26 strains available for genotyping: RA (8 tem-1 positive isolates); RB (5 tem-1 and 8 oxa-1-positive isolates); RC and RD (four and one oxa-1-positive isolates).

Conclusions: Our findings indicate that the high prevalence of A/C-resistance among *K. pneumoniae* isolates resulted from the spread of three different epidemic clones and was mediated by the production of two kinds of penicillinases poorly inhibited by clavulanate (i.e. OXA-1 and inhibitor resistant enzymes, likely of IRT type), a fact that could be related to the elevated consumption of A/C in the centre. Interestingly, the present results suggest that oxa-1 might be associated with aac(6′)-1b, and perhaps other resistance genes, within a class 1 integron.

P1227 Differentiation between BRO-1 and BRO-2 β-lactamases in clinical isolates of *Moraxella catarrhalis* collected 2002–2003

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Objectives: To determine the presence and incidence of the bro1 and bro2 β-lactamase genes in clinical isolates of *Moraxella catarrhalis* collected in the winter of 2002–2003.

Methods: A novel PCR RFLP method was developed and used to screen 1,449 isolates of *M. catarrhalis* for the presence of either the bro1 or bro2 genes. This was achieved by initially developing primers that hybridised to conserved promoter sequence regions upstream of both genes (BROF – 5′-TRGTGAAGTGATTTTKRRMTTG-3′ BROR – 5′-GCAATTTATTAACTGGATGTA-3′), yielding PCR products differing in size by 21bp (bro1 = 165bp and bro2 = 144bp).

After PCR thermocycling, the resultant amplicon was digested using the restriction enzyme tsp509i, yielding visible fragments of 55bp and 91bp and 91bp alone for bro1 and bro2, respectively. The PCR RFLP products were visualised on a 3.5% agarose gel. During the analysis, sequencing was performed on 2 PCR products from isolates that showed anomalies in their PCR amplification products.

Results: Of the 1,449 isolates tested 1,314 (91%) were positive for bro1 and 65 (4.2%) positive for bro2. Seventy (70) isolates (4.8%) were negative for both genes. A single bro1 isolate had a single nucleotide polymorphism (SNP) at position T30C of the promoter region yielding a PCR RFLP product of 146bp, whilst a second bro1 isolate yielded 3 visible bands of 31bp, 91bp and approximately 150bp.

Conclusions: Consistent with published data, bro1 was found to be the most common β-lactamase type present within our global, clinical *M. catarrhalis* isolates at an incidence of 91%. Also consistent with published data, 5% of our isolates possessed the bro2 gene. Only 1

isolate was identified that possessed nucleotide polymorphisms for the restriction enzyme used, mirroring the low frequency of mutation within the promoter regions of bro1 genes. It was found that a PCR RFLP methodology yielded more accurate results regarding the identification of bro1 and bro2 over PCR alone.

Quinolone and fluoroquinolone resistance – Part 1

P1228 Topoisomerase alterations and ciprofloxacin resistance in clinical isolates of *Klebsiella pneumoniae*

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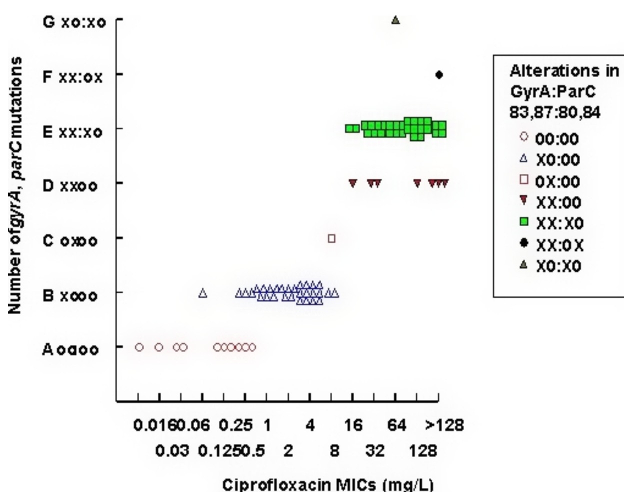
Objectives: To determine the frequency and distribution of amino acid alterations in the quinolone binding region (QBR) of GyrA and ParC and the effect of these changes in ciprofloxacin susceptibility amongst 78 clinical isolates of *Klebsiella pneumoniae* showing ciprofloxacin reduced susceptibility or resistance.
Methods: Sequencing of PCR derived amplicons from a 626bp region of gyrA, and a 388bp region of parC covering the extended quinolone resistance-determining regions (QRDR).



Results: There were 19 different combinations of topoisomerase variants amongst the 78 isolates. The most common mutation, in 97% of mutants, occurred at codon 83 in gyrA, mostly resulting in either S83Y or S83F substitutions in GyrA. There was a strong correlation (r=0.90, 95% CI 0.84–0.93) between the number of target alterations (particularly in GyrA) and ciprofloxacin MICs. No target alteration was found in the 6 isolates with only small reductions in susceptibility. 70/78 isolates with greater reduced susceptibility had target alterations: 29 had alterations in S83, and 1 in D87, of GyrA; 39 of the other 40 had different combinations of double or triple alterations in GyrA and ParC; one had S83Y and F147Y alterations in GyrA and ParC, respectively. The figure shows the association between topoisomerase gene mutations and ciprofloxacin MICs. In this figure a circle=isolates with wild-type sequence in QRDR of both gyrA and parC; triangle=single gyrA mutation; open square=single gyrA mutation at codon D87; inverted triangle=gyrA double mutations at S83 and D87; solid square=gyrA double mutations at S83 and D87 and parC single mutation at S80; solid circle=S83F and D87N in GyrA and E84K in ParC; solid triangle=one mutation in each of gyrA and parC. 00:00=wild type for S83 and D87 in GyrA and S80 and E84 in ParC in the QBR; X0:00=change at S83 in GyrA but not in ParC; 0X:00= change at D87 in GyrA but not in ParC; XX:00=changes at S83 and D87 in GyrA and not in ParC; XX:X0=changes at S83 and D87 in GyrA and at S80 in ParC; XX:0X= changes at S83 and D87 in GyrA and at E84 in ParC; X0:X0=changes at S83 in GyrA and at S80 in ParC.
Conclusion: Mutations in the QRDR of gyrA play a major role in ciprofloxacin resistance in these isolates and the degree of resistance is related to the number of mutations. Double GyrA alterations alone can produce highly resistant ciprofloxacin phenotypes.

P1229 Prevalence of efflux pump activity and its role in ciprofloxacin resistance in clinical isolates of *Klebsiella pneumoniae*

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Objectives: To determine the prevalence of efflux pump activity and its contribution to ciprofloxacin resistance in clinical isolates of *Klebsiella pneumoniae*.
Methods: Drug accumulation studies were performed on 26 ciprofloxacin susceptible and resistant isolates in early log-phase using [14^{C}] ciprofloxacin with and without the proton motive force dissipater carbonyl cyanide 3-chlorophenylhydrazone (CCCP). The total accumulation time studied was 20 minutes; CCCP was added at 10 minutes. The efflux index, a measure of efflux activity, was the ratio of the amount of drug accumulated at the 18th minute to the amount accumulated at the 4th minute. Membrane-associated reduced influx-mediated resistance (MARIM-R) was calculated by dividing the difference in drug accumulation at the 10th and the 4th minutes (in the absence of CCCP) by the amount of drug accumulated at the 4th minute. Values of >1.5 were significant.
Results: (Table). 92% of tested isolates were positive for efflux. The contribution of efflux to the removal of cell-bound ciprofloxacin ranged from 50% to 90% of total cell-associated drug. In some highly resistant isolates efflux pump activity extruded 90% of the drug. Efflux activity was present in all isolates with ciprofloxacin MICs >1 mg/L and contributed to reduced ciprofloxacin susceptibility in isolates that had no target site alteration. High-level activity usually occurred in isolates with high ciprofloxacin MICs, but these isolates usually had double GyrA alterations as well. None of the 26 isolates had MARIM-R.

Isolate #	CIP MIC mg/L	Efflux index	Efflux mediated reduction in drug accumulation (%)
1	0.016	1.8	46
2	0.032	2.1	44
3	0.064	5.4	81
4	0.25	1.7	49
5	0.5	1.3	6
6	0.5	5.1	79
7	0.125	1.3	30
8	1	5.4	82
9	1	6.8	86
10	2	4.4	79
11	4	2.3	58
12	4	4.1	76
13	4	4.5	69
14	4	5.2	77
15	8	2.0	55
16	8	7.6	87
17	128	6.8	85
18	128	9.0	89
19	>128	7.7	88
20	16	3.0	67
21	32	10.8	89
22	64	5.8	83
23	64	6.1	82
24	128	3.4	71
25	>128	3.5	72
26	>512	8.4	87

Conclusion: This study demonstrates that efflux activity is very common in *K. pneumoniae* and contributes to decreased ciprofloxacin susceptibility even in the absence of topoisomerase alterations. MARIM-R was not detected. Efflux activity may play a role in initial selection of isolates with reduced ciprofloxacin susceptibility, which then survive when ciprofloxacin is used. Surviving strains may then accumulate target site alterations and higher pump activity, leading to increasing ciprofloxacin resistance.

P1230 Mechanisms of fluoroquinolone resistance in *Salmonella enteritidis*

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Objectives: To determine the contribution of target gene mutations and efflux pump activity to nalidixic acid resistance in *S. enteritidis* field isolates (104 and 5408) and their in-vitro selected ciprofloxacin resistant mutants (104-cip and 5408-cip). The fitness cost associated with high-level fluoroquinolone resistance was also studied.
Methods: Disk diffusion and MIC testing were performed in the presence and absence of the efflux pump inhibitor Phe-Arg-beta-Naphthylamide (PABN). Ciprofloxacin accumulation was measured fluorometrically. Expression of the efflux transporter acrB, the global regulators soxS, marA, ramA and rob and the outer membrane porin ompF were measured by real-time RT-PCR. Invasiveness of ciprofloxacin resistant mutants was assessed in Caco-2 intestinal epithelial cells.
Results: High-level nalidixic acid resistance was associated with an Asp87Tyr mutation in gyrA. This resistance was partially reversed by PABN. Acquisition of high-level ciprofloxacin resistance resulted in additional mutations in gyrA, gyrB and parE. Ciprofloxacin-resistant mutants accumulated less ciprofloxacin than parent strains and demonstrated a multi-drug resistant phenotype attributable to enhanced efflux activity. Both mutants showed increased expression of acrB and the global regulator marA and a differential expression of soxS and ramA. Increased expression of acrB was not associated with mutations in its local regulator acrR. A mutation was found in the helix-turn-helix region of soxR in 104-cip that showed increased soxS expression. No mutations were found in any of the other global regulators investigated. Mutants formed smaller colonies and demonstrated slower growth rates than their parental strains. Mutants were significantly less invasive than the parents in Caco-2 cells. Electron microscopy revealed altered morphology of the mutants.
Conclusion: Both target mutations and efflux pump activity contribute to quinolone resistance in *S. enteritidis*. Increased expression of acrB may result from differential expression of global regulators. High-level ciprofloxacin resistance is associated with a fitness cost.

P1231 Virulence genotypes of *Escherichia coli* strains of human and avian origin in relation to ciprofloxacin resistance status

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Objectives: Previous studies have suggested that human fluoroquinolone resistant (FQr) *Escherichia coli* strains probably emerged as a consequence of fluoroquinolone use in poultry. This study aims to provide further insight on the possible avian origin of human FQr extra-intestinal pathogenic *E. coli* (ExPEC) strains.
Methods: The presence of 12 virulence factor (VF) genes (agg, eae, cldt, cnf, cvaC, ireA, iroN, iss, iucD, iutA, traT, tsh) commonly associated with human and/or avian ExPEC isolates was investigated by PCR in 125 *E. coli* isolates from extra-intestinal infections (61 susceptible and 64 ciprofloxacin resistant, respectively) and 113 isolates from chicken and turkeys (47 susceptible and 66 ciprofloxacin resistant, respectively). Genetic relatedness among 40 human and avian *E. coli* strains belonging to the traT-iucD-iutA virulence profile was assessed by PFGE.
Results: Irrespective of ciprofloxacin susceptibility, human isolates showed a significantly lower prevalence of cvaC, iroN, iss and tsh genes and a significantly higher prevalence of cnf compared to avian strains. Stratifying by ciprofloxacin susceptibility, susceptible human and avian isolates differed significantly for distribution of cnf, cvaC, iroN, iss, iucD, iutA and tsh genes, being all, except cnf, more frequent in avian isolates. Resistant human isolates differed from resistant avian isolates for distribution of 5 VF genes: iroN, iss, tsh (predominant in avian strains), iucD and iutA (predominant in human strains). Both human and avian FQr isolates harboured fewer VF genes than susceptible isolates. The two major VF profiles of the human isolates were traT-iucD-iutA and iucD-iutA, (65.6% of the resistant and 13.1% of the susceptible isolates). These two profiles were detected in a few resistant avian strains (traT-iucD-iutA, 9.1% and iucD-iutA, 3%). Conversely the two major profiles of avian strains (traT-cvaC-iucD-iroN-iss-tsh-iutA and traT-iroN-iss) did not occur among human isolates. By PFGE, an extensive genetic diversity was observed between the human and avian isolates belonging to the same traT-iucD-iutA profile.
Conclusion: Our results confirm previous data indicating strong differences in virulence genotype between ciprofloxacin susceptible and resistant human ExPEC strains. However, the resistant human isolates were distinct from both susceptible and resistant avian isolates and the results therefore do not support their avian origin.

P1232 Phylogenetic background of *Escherichia coli* resistant to quinolone and fluoroquinolone

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Objective: To assess how resistance to quinolone (Q) and fluoroquinolone (FQ), and production of extended-spectrum β-lactamases (ESBLs) relates to the phylogenetic background of clinical *Escherichia coli* isolates.
Methods: A total of 337 *E. coli* strains, isolated from clinical samples were selected. The first 222 strains were collected consecutively after the susceptibility test, which allow selecting 120 strains Q and FQ susceptible (SS), 36 Q resistant and FQ susceptible (RS) and 66 Q and FQ resistant (RR). Further strains RS and RR were selected consecutively, till completed a total of 105 and 112 respectively. Resistance to nalidixic acid and ciprofloxacin was tested by disc diffusion method according to CLSI and presence of ESBLs by synergy test between clavulanic acid and third generation cephalosporins. Phylogenetic group was performed by a triplex PCR.
Results: The prevalence of Q resistance was 45.9% (102/222), FQ 29.7% (66/222), and 4.6% (11/222) produced ESBLs. Among the total of 337 isolates, the most prevalent phylogenetic group was B2 with 177 strains, followed by group D with 68, group A with 49 and B1 with 43. *E. coli* SS and RS showed a similar distribution among phylogenetic groups (66.7% and 60.9% in group B2, 15% and 20% in D, 11.7% and 8.6% in A and 6.7% and 10.5% in B1). However RR strains exhibited a shift to non-B2 groups: 23.2% derived from group A, 21.4% from B1, 25.9% from D and 29.5% from B2, (RR vs. RS $P<0.01$ in groups A, B1, B2; RR vs. SS $P<0.01$ in groups B1, B2, D). Of the 337 *E. coli* studied, 20 produced ESBLs: 1 strain SS, 6 RS and 13 RR ($P<0.05$ in SS vs. RS and SS vs. RR). ESBLs producers exhibited a similar distribution among phylogroups A, B1 and D (8.2, 11.6 and 10.2%); whereas were quite less prevalent in pathogenic group B2 (2.3%; $P<0.05$).
Conclusion: *E. coli* resistant to Q mainly derived from phylogenetic group B2, whereas isolates resistant to both Q and FQ exhibited shifts towards groups A, B1 and/or D. This fact led us to suspect that these two subgroups represent distinct populations, RS isolates could have derived predominantly from susceptible *E. coli* group B2 (possibly within a human host), whereas RR isolates from susceptible *E. coli* group A isolates (possibly within an animal host), both in the context of drug exposure. The depletion for phylogenetic group B2 among ESBLs producers suggest a similar phenomenon.

P1233 **Genetic relatedness between ciprofloxacin-resistant *Salmonella* Kentucky isolated from humans and poultry meat**

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Objectives: To compare *Salmonella enterica* serotype Kentucky strains (*Salmonella* Kentucky) resistant to ciprofloxacin isolated in France from travellers returning from Morocco with those from raw poultry meat produced in Morocco.

Methods: *Salmonella* Kentucky strains from poultry meat (n = 3) have been identified between December 2006 and June 2007 during a food safety survey conducted since 2004 in Pasteur Institute from Morocco. Strains from human origin (n = 3) have been isolated from travelers returning from Morocco (2006: n = 3; 2007, n = 3), and from a patient hospitalised in the Ibn Rochd Hospital (Casablanca) in 2006. Nalidix acid and ciprofloxacin MICs were performed by E-Test. To identify the mechanisms of resistance to ciprofloxacin, the quinolone-resistance determining regions of gyrA and parC were sequenced and the presence of plasmid-mediated quinolone resistance genes (qnrA-B-S, aac(6′)-Ib-cr) were tested for all isolates. All isolates were genotyped by pulsed-field gel electrophoresis (PFGE) with XbaI restriction following PulseNet's running conditions.

Results: Among the 13 strains, 9 (poultry meat, n = 3; patients, n = 3) were resistant to nalidixic acid and to ciprofloxacin (MIC > 16 mg/l, >1 mg/l respectively) and have the same amino-acid substitutions within gyrA and parC (gyrA: Ser83-Phe, Asp87-Asn; parC: Ser80-Ile). Within parC, an additional substitution (Thr57-Ser) was observed. This substitution, however, did not appear to be associated with quinolone resistance because it was also identified in nalidixic acid-susceptible isolates. All strains were negative for plasmid mediated quinolone resistance genes. Among quinolone-resistant isolates, 2 PFGE patterns were displayed: the first one (P1) was observed in all poultry isolates and 4 human isolates; the second one (1 traveler isolate) shared 80% identity with P1. For quinolone-susceptible strains, 3 PFGE patterns were displayed and shared less than 50% identity with previous PFGE profiles.

Conclusions: this study showed clearly the close genetic relatedness among ciprofloxacin-resistant *Salmonella* Kentucky isolates and identified poultry meat as a probable source for human contamination. Moreover, it can be assumed that an unique ciprofloxacin-resistant *Salmonella* Kentucky has spread all over different poultry flocks in the Casablanca's region.

P1234 **Epidemiology of elevated fluoroquinolone MICs of *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* from the GLOBAL Study (1999–2005)**

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Objective: Isolates (ISO) were collected during 1999, 2002, 2003, and 2005 as part of the GLOBAL study, during which 360 *S. pneumoniae* (SP) showed levofloxacin (LFX) MICs ⩾2 mg/L by broth microdilution. Serotyping profiles of these SP were determined in this study. Previous experience has shown that in studies where LFX resistance rates were in excess of 1% that it was often the result of an outbreak of clonal ISO. To address the relatedness of SP ISO from 2005 with elevated LFX MICs, pulsed-field gel electrophoresis (PFGE) was performed and related to QRDR profile and serotype (ST). In addition, 8 ISO of H. influenza (HI) and *M. catarrhalis* (MC) with elevated LFX MICs (⩾0.05 mg/L) were subjected to PFGE analysis to determine relatedness, if any.

Methods: ISO were centrally tested by broth microdilution (CLSI; M7-A7) and interpreted according to CLSI M100-S16 and M45-P (MC). 84 SP with MICs ⩾2 mg/L were STed using standard methodology. ISO of SP, HI, and MC from 2005 with elevated LFX MICs underwent PFGE. ISO with ⩾90% similarity in PFGE pattern were considered related. SP were further characterised by sequencing the quinolone resistance determining regions (QRDRs) of gyrA, gyrB, parC, and parE.

Results: Among the 360 SP with elevated LFX MICs from 1999–2005, 44 STs were identified and STs 23F, 14, and 19F were the most common (>10% each). Among the 2005 SP ISO (N=86), STs 8, 19F, and 23F were detected at >10%. Of these, there were 5 genetically related groups detected by PFGE (1 centre in Spain (ES) [n = 3], 2 separate centres in ES [n = 34], 1 centre in Hong Kong (HK) [n = 3], a separate centre in HK [n = 3], and 1 centre in Germany (DE) [n = 3]). Similar STs and QRDR profiles were observed within each group. The remaining SP ISO showed little genetic similarity with a wide distribution of STs. Little genetic relatedness was observed among ISO of HI and MC with elevated LFX MICs.

Conclusion: Among the SP from 2005 with elevated MICs to LFX, 5 clusters of genetically related ISO were detected suggesting outbreaks of certain lineages of this phenotype within centres in ES, DE, and HK. The remaining SP ISO were of variable ST, PFGE patterns and QRDR profiles. Few HI and MC ISO with elevated LFX MICs were detected in 2005, and no genetic relatedness was apparent among these ISO. These results illustrate the importance of utilising both surveillance and molecular epidemiological studies for monitoring the emergence and spread of resistance.

P1235 **Molecular analysis of fluoroquinolone resistance in *Streptococcus pneumoniae* in Turkey**

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Objectives: Fluoroquinolones(FQs) are recommended drugs for the treatment of *Streptococcus pneumoniae* infection which increase the risk for the development of resistance. FQ resistance occurs primarily by mutations in quinolone resistance determining regions(QRDRs) of parC, gyrA, parE and/or gyrB genes. The aim of this study was to evaluate fluoroquinolone-resistant *S. pneumoniae* in multicentre isolates from Turkey to investigate the mechanisms of resistance.

Methods: A total of 288 out of 297 study isolates with MIC⩾1g/L for Levofloxacin(LEV) were amplified by PCR for the QRDRs of gyrA, parC and parE. Restriction fragment length polymorphism (RFLP) by HinfI, MboII, LweI digestions of PCR products was carried out to identify mutations known to confer FQ resistance. Isolates with mutations by PCR-RFLP analyses were confirmed by DNA sequence analysis. MICs were also determined for penicillin(PEN), ceftriaxone(CRO), erythromycin(EM) and clindamycin(CD) by broth microdilution using CLSI methods. Serotyping was performed using sequential multiplex-PCR and quellung reaction with capsular antisera for *S. pneumoniae*.

Results: Twenty-four(8.1%)*S. pneumoniae* strains were levofloxacin nonsusceptible(MIC ⩾4g/L) and antimicrobial susceptibilities of these isolates are presented in Table 1. Fifteen different serotypes were recognised. Eleven strains had mutations in QRDR of parC (K137N(n = 3) and S79Y, D83N(n = 3)). Eighteen isolates had parE mutations (I460V(n = 38) and D435N(n = 3)). All isolates with MICs of 4g/L contained no mutations in gyrA, parC or parE that are known to confer resistance to FQs. Although there were eight isolates with MIC ⩾8 g/L, only three of levofloxacin-resistant isolates analysed possessed a gyrA mutation (S81F). Two of these strains had additional mutations in parC(S79Y,D83N) and one strain had the D435N mutation in parE known to confer resistance. They all had different serotypes belonging to 34, 23F and 6B.

Table 1. Antimicrobial susceptibilities of 24 FQ-resistant isolates

	MIC_{50}	MIC_{90}	Range	I (%)	R (%)
LEV	4	>8	4–>8	16 (66.7)	8 (33.3)
PEN	⩽0.06	>2	⩽0.06–>2	6 (25.0)	5 (20.8)
CRO	⩽0.25	2	⩽0.25–2	5 (20.8)	–
EM	⩽0.25	>8	⩽0.25–>8	–	7 (29.2)
CD	⩽0.25	>8	⩽0.25–>8	–	6 (25.0)

Conclusion: FQ nonsusceptibility was observed in 8.1% of isolates in this study. Mutations known to confer resistance to FQs were not observed in strains with MICs of 4g/L. Three of eight strains showing higher levels of resistance contained mutations in the QRDRs of gyrA, parC and parE, however 5 strains were either wild-type or contained mutations which do not confer resistance. This data suggests that perhaps other mechanisms of resistance to the FQs are present in these strains such as efflux or mutations outside the QRDR region.

P1236 Interactions between serotype and mutations in quinolone-resistance determining regions of *Streptococcus pneumoniae*

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Background: Mutations in QRDR regions are the primary cause of fluoroquinolone resistance (FQR) in *S. pneumoniae* (SPN). It is not known whether the distribution of mutations and/or the association of QRDR with increasing MIC is similar in different SPN serotypes (STs).
Methods: The Canadian Bacterial Surveillance Network (CBSN) has been monitoring trends in antimicrobial resistance in SPN in Canada. From 1995–2006, 27,951 isolates from across Canada have been collected. Serotyping and sequencing of the gyrA and parC regions was performed for all FQR isolates FQ (N=451), and a sample of others (N=3438).
Results: The most common parC mutations were: K137N (N=870), S79F (293), S79Y (106), D83N (36), D83Y (17), D83G (9), S52G (6), N91D (6), R95C (5), Y129S (5), D78N (4), S79A (2), A115P (2). 14 other mutations were identified once. S79F, S79Y, D83N/Y/G, D78N/A, and A115P were associated with increased MICs to FQ; other mutations were not. K137N was present in <5% of isolates of SGs 3, 4, 10, 11, 15, 18, 20, 22, 33, 35, 38, >85% of isolates of SGs 12 and 9, and an intermediate percentage of other SGs. Among FQR, S79Y is significantly more common in isolates of ST 22F, 6A and 6B, and mutations at position 83 are more common in ST 9N and 9V ($P < 0.01$). Of isolates with parC mutations, those of ST 12F had significantly lower MICs to all FQ than those of other STs. 27% of FQS SPN with a parC mutation at position 79 were ST 12F, compared to 3% of FQR isolates. The most common gyrA mutations were S81F (N=179), S81Y (33), E85K (33), S114G (14), E85G (4). 12 other mutations were identified once. Only mutations at position 81 and 85 were associated with increased FQ MICs. The prevalence of gyrA mutations in the presence of parC mutations varied significantly with ST; eg. 10/12 (83%) 19A and 32/40 (80%) 19F strains with a parC mutation also had a gyrA mutation, compared to 9/24 (38%) ST 3 and 6/17 (35%) ST 12F ($P < 0.001$). When isolates were categorised by whether or not their serotypes were included in 7- or 23-valent pneumococcal vaccines, there were no significant differences in the prevalence of parC mutations. Isolates of STs in the 23-valent vaccine with a parC mutation were somewhat less likely to have a gyrA mutation than other isolates (66% vs 55%, $P = 0.07$).
Conclusion: Mutations in QRDR determining regions occur with differing frequencies in isolates of different ST. Changing ST distributions associated with pneumococcal vaccination programs may have an impact on FQR rates.

P1237 Identification of SmrA, a novel efflux pump involved in fluoroquinolone resistance in *Streptococcus suis*

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Objectives: *Streptococcus suis* is a world-wide distributed pathogen with increasing prevalence in humans. It has recently been reported as the cause of high mortality outbreaks in Southeast Asia among the population in close contact with infected pigs or contaminated food.
During the last years rising fluoroquinolone resistance among Spanish isolates collected in our laboratory has been observed. We have recently described the influence in this phenotype of mutations in gyrA and parC, the genes encoding the drug's targets (Escudero et al. AAC 2007 51:777–82). Interestingly, the MIC of some strains was reduced in presence of reserpine pointing to the coexistance of a drug efflux phenomenon.
Methods: Strains identification was PCR based. MICs were carried out following CLSI guidelines. PFGE was performed using ApaI endonuclease. Sequencing of PCR products was performed by Secugen S.L. 4Peaks, CLC workbench, DNA Strider and Phyre (www.sbg.bio.ic.ac.uk/phyre/) were used for sequence analysis and protein modeling.
Results: *S. suis* available genomes were scanned for the presence of putative efflux pumps. A gene encoding a 401 aminoacid protein sharing 58% homology with PmrA, an efflux pump related to fluoroquinolone resistance in *S. pneumoniae*, was identified and named SmrA (*suis* multidrug resistance). Protein modeling of SmrA, revealed 12 transmembrane segments and elicited to assign this putative pump to the Major Facilitator Superfamily (MFS). Complete nucleotide sequence of smrA including its promoter region was determined from five fluoroquinolone susceptible clinical isolates, three high level resistant and two intermediate level resistant strains. PFGE of all strains revealed no genetic link between all isolates. Sequencing of these regions revealed the presence of mutations leading to a critical aminoacid substitution in position 107 (V/T107A) in all five resistant clinical isolates. Furthermore the three high level resistant isolates bore mutations in the putative −10 box, indicating that, as for other MFS members, protein structure together with expression levels are involved in SmrA mediated fluoroquinolone resistance in *S. suis*.
Conclusion: SmrA is a novel member of the Major Facilitator Superfamily and plays a role in fluoroquinolone resistance in clinical *S. suis* isolates. Acquisition of this efflux phenotype seems to be a stepwise phenomenon involving mutations in the promoter region as well as in the structural gene of SmrA.

P1238 Epidemiological analysis of fluoroquinolone-resistant group B streptococci

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Objectives: We reported increasing number of fluoroquinolones (FQ)-resistant group B streptococci (*S. agalactiae*; GBS) in Japan since 2000. FQ-resistant GBS was isolated from neonates and their mothers and elderly patients with underlying urogenital diseases or hematological malignancies under long-term administration of FQ. Analysis of the mechanism of FQ-resistance in addition to epidemiological studies was performed to reveal whether FQ-resistant GBS was genetically related.
Methods: Clinical and epidemiological data were obtained from patients with GBS isolated in our 1300-bed tertiary hospital between October 2005 and January 2007. FQ-resistance was judged with MIC in case of $>8\,\mu g/ml$ for levofloxacin. Serotypes of GBS were determined by antisera (Denka Seiken, Tokyo). The nucleotide sequences of the quinolone resistance-determining region (QRDR) including of gyrA gene and parC gene were determined. Pulsed-field gel electrophoresis (PEGE) analysis was performed on GBS strains.
Results: From 84 patients, 91 GBS were isolated from 49 genitourinary, 35 respiratory, 4 tissue, 2 blood, one peritoneal specimens. FQ-resistant GBS were 21 from 17 (20%) patients. All FQ-resistant GBS except one (94%) was serotype Ib, whereas FQ-sensitive GBS belonged to various serotypes (VII, V, Ia, Ib, III, II, VIII, and NT). FQ-resistant GBS had the same triple point mutations within QRDR; In gyrA gene, Ser-81 was changed to Leu (TCA-TTA) in 11/11 strains, and in parC gene Ser-79 was changed to Phe (TCC-TTC) and Ile-81 had a silent mutation (ATC-ATT) in 10/10 strains. PFGE on 16 FQ-resistant GBS revealed 4 major groups with high relatedness, although FQ-sensitive GBS showed slightly related PFGE patterns.
Conclusion: Most of all FQ-resistant GBS was serotype Ib and shared identical mutations in the QRDR and high related PFGE patterns. These findings suggest that the closely related FQ-resistant GBS had been possibly emerged and disseminated in the community in recent years, as receiving the antibiotic pressures by quinolone administration.

P1239 **Molecular characterisation of quinolone-resistance in erythromycin-resistant *Streptococcus agalactiae* clinical isolates**

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Objectives: *Streptococcus agalactiae* (GBS) remains susceptible to penicillin; nevertheless, an increase in the resistance to other antimicrobial families, such as macrolides and more rarely fluoroquinolones, has been described. The purpose of the study was to surveillance the fluoroquinolone resistance in GBS isolated from blood cultures, in erythromycin-resistant GBS isolated from vaginal colonisation and characterise the molecular mechanisms of resistance implicated and their genetic similarity.
Methods: Antimicrobial susceptibility (according to CLSI guidelines) of 153 erythromycin-resistant GBS from vaginal colonisation (2002–2004) and 28 GBS from blood cultures (1994–2004) were recorded by our Clinical Laboratory (Hospital Lozano Blesa, Zaragoza, Spain). Levofloxacin and ciprofloxacin resistance in GBS was identified by disk diffusion and broth microdilution methodology in all the isolates. Therefore, we determined the nucleotide sequences and deduced amino acid sequences of gyrA and parC genes including QRDRs in 5 isolates with low-level susceptibility to levofloxacin. These sequences were compared with those of susceptible strains. Pulsed-field gel electrophoresis (PFGE) analysis was performed on the 5 GBS isolates following Sma1 digestion.
Results: Five low-level fluoroquinolone-resistant GBS strains were detected (MICs range were 0.5–2 mg/l) but no high-level fluoroquinolone-resistant GBS (MICs >8 mg/l) were found in the GBS isolates. Of these, in 3 isolates no mutations in the QRDR of parC were found, in a blood isolate collected from a patient previously treated with norfloxacin the sequence analysis of the parC gene showed a base pair chain TCT/CCT at position 238 that resulted in an aminoacid substitution Ser80Pro and in a vaginal isolate three parC silent mutations were found and their implications in quinolone resistance remains unclear. No mutations in the QRDRs of gyrA were detected. The PFGE pattern study did not suggest genetic relation of these five isolates.
Conclusion: Low-level fluoroquinolone-resistant GBS were recovered from 5 patients and we found that one contained an aminoacid substitution in the topoisomerase IV gene (Ser80Pro). This mutation was described previously only in *Streptococcus pyogenes*. It seems that parC is the primary target of the quinolones in *S. agalactiae*. These findings emphasise the importance of monitoring antibiotic susceptibility to quinolones antibiotics.

PK/PD: clinical studies

P1240 **Pharmacokinetics of azithromycin in plasma and sinus tissue after administration of extended release and immediate release formulations in chronic rhinosinusitis patients**

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Objectives: High drug levels at an infection site are desirable for optimal activity. The aim of this study was to determine if a single dose of azithromycin (AZ)-extended release (ER) (2 g) achieves higher AZ levels in sinus tissue compared to AZ-immediate release (IR) (500 mg daily for maximum of 3 days).
Methods: This was a randomised open-label multi-centre study. Seventy-one chronic rhinosinusitis patients received either a single dose of 2-g AZ-ER or AZ-IR 500 mg once daily dose for 1–3 days. Sinus tissue and plasma samples were collected at the time of endoscopic surgery, which was 2, 4, 6, 8, 12, 24, 48, 72, 96, 120, 144 and 168 hr after the first AZ dose, 4 subjects per time point. AZ levels were analysed by LC/MS/MS method and averaged at each time point. The resultant composite profile was used for Pharmacokinetics (PK) analyses by a non-compartmental method.
Results: Sixty-eight patients completed the study. The patients aged 19–81, with majority being white (90%) and males (59%). 53 subjects were evaluable for PK analysis.
After the administration of both formulations, AZ was absorbed and distributed quickly in systemic circulation. Sinus tissue concentrations were measurable at 168 hr from both formulations. PK parameters are shown in the table.

Matrix	Parameter	AZ-ER (n = 24)	AZ-IR (n = 29)	ER/IR Ratio
Plasma	C_{max} (ng/mL)	364	112	3.3
	AUC_{24} (ng·h/mL)	4865	933	5.2
	AUC_{168} (ng·h/mL)	10994	N/A	
Sinus tissue	C_{max} (ng/mg)	12.2	1.44	8.5
	AUC_{24} (ng·h/mg)	137	19.7	7.0
	AUC_{168} (ng·h/mg)	684	N/A	

N/A = not applicable.

Comparison between formulations: Within the first 24 hr, AZ exposure from ER was 5.2 and 7.0 fold higher than IR in plasma and sinus tissue respectively. This is consistent with the dose from ER being 4 times higher than IR.
Comparison between matrices: For ER formulation, AUC_{24} and AUC_{168} in sinus tissue were 28.2 and 62.2-fold higher than in plasma, which indicates that once AZ enters the sinus tissue, it remains for a prolonged duration. For IR formulation, AUC_{24} in sinus tissue was 21.1-fold higher than AUC_{24} in plasma, which is similar to the ER penetration ratio.
Conclusions: AZ demonstrated good penetration into sinus tissue regardless of formulation. However, dosing with AZ-ER (2 g) resulted in an increased exposure compared to the first dose of 500 mg IR within the first 24 hr after start of therapy.

P1241 **Penetration of voriconazole into human tissue**

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Objectives: Voriconazole (VRC) is a second-generation triazole with a broad antimycotic spectrum. VRC exhibits a good penetration into body fluids, such as cerebrospinal fluid, epithelial lining fluid and pleural effusion. Data on VRC distribution into human tissue after administration of VRC have been sparse so far. VRC tissue levels were determined in tissue samples obtained during routine autopsy from patients who have died during treatment with VRC.
Methods: VRC levels were measured in various samples derived from different tissues (kidneys, liver, heart, spleen, lung, brain) of 2 patients, who had been on treatment with VRC. Patient 1 had received a single dose of 200 mg, patient 2 had obtained 200 mg/day for 10 days. The intervals between the last administration and death were 36 and 12 hours, respectively. Both patients had been on vasopressor therapy and mechanical ventilation. Antimycotic therapy had been initiated for suspected or proven invasive fungal infections. VRC tissue levels were assessed by extraction of homogenised tissue samples, purification by solid phase extraction and measurement of VRC by high performance liquid chromatography.
Results: In VRC treated patients, highest VRC concentrations (mean ± standard deviation) have been found in the kidneys (patient 1: 1.97±0.41 μg/mL, patient 2: 6.89±0.06 μg/mL) and liver (patient 1: 2.14±0.40 μg/mL, patient 2: 4.21±0.77 μg/mL). Tissue levels of VRC in patient 2 exceeded those achieved in patient 1 in all samples. In different areas of brain tissue VRC was below the limit of detection (<0.25 μg/mL) in patient 1 and achieved levels of 3.34±0.18 μg/mL in patient 2. The levels were comparable in cortex, hippocampus, nucleus caudatus, medulla oblongata and cerebellum. VRC concentrations amounted 1.31±0.03 μg/mL and 2.95±0.05 μg/mL in samples of the spleen of patient 1 and 2, respectively. In the heart samples of

patient 1 VRC was undetectable, but reached a mean concentration of 2.44±0.25 μg/mL in patient 2. In all lung samples VRC was measurable and reached mean levels of 1.30±0.63 μg/mL (patient 1: 0.74±0.02 μg/mL, patient 2: 1.87±0.27 μg/mL).
Conclusion: VRC appears to penetrate rapidly into human tissues. VRC accumulates in the liver and the kidneys. Its penetration into brain and heart is probably slower.

P1242 Pharmacodynamics of vancomycin against MRSA: implications of bacterial inoculum for the clinical breakpoint

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Background: Vancomycin (V) is gold standard therapy for MRSA infection, yet despite wide clinical usage the clinical breakpoint to define susceptible *S. aureus* remains controversial (CLSI $S \leqslant 2$ mg/L; EUCAST $S \leqslant 4$ mg/L). We used an in vitro pharmacokinetic (PK) model to define AUC/MIC targets for various V antibacterial effects (ABE) against MRSA and used these in Monte Carlo simulations using patient PK data to define various clinical target attainment rates (TAR).
Methods: An in vitro PK model was used to perform a dose ranging study with two representative UKEMRSA 15 or 16 strains at inoculum of 10^6 or 10^8 CFU/ml. The AUC/MIC range was 0–2500. Total V AUC/MIC for 24 h static, 1, 2, 3 or 4 log drop in bacterial count were established for each inoculum using a Sigmoid Emax model. Patient V AUC 24 (mg/L.h) was derived from a population PK model developed for V. Monte Carlo simulations were used to define TAR using patient AUC values and AUC/MIC targets from the PK model.
Results: At an inoculum of 10^6 CFU/mL the total drug AUC/MIC required at 24 h for a static effect was 34: 1 log drop 66; 2 log drop 254; 3 log drop 303 and 4 log drop >2500. At an inoculum of 10^8 CFU/ml the AUC/MIC for a 1 log drop at 24 h was 426, 2 log drop 588 and 3 log drop >2500. Patient AUC 24 (mg/L.h) was mean 472±221 (min 155, max 1306, n = 50 subjects). A total V AUC/MIC target associated with 0–1 log drop (initial inoculum 10^6 CFU/ml) resulted in a TAR of >90% at an MIC 4 mg/L. The AUC/MIC targets associated with a similar ABE at 10^8 CFU/mL resulted in a TAR of >80% at MICs of 0.5 mg/L.
Conclusion: MRSA inoculum has a significant effect on TAR for V. At an inoculum of 10^6 CFU/mL a clinical breakpoint for V of 4 mg/L is justified; at 10^8 CFU/ml inoculum a breakpoint of 0.5 mg/L is more appropriate.

P1243 Identification of mutations in HERG and KCNE2 genes associated with arrhythmia in patients treated with macrolides

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Several new antibiotics have been whitedrawn from the market because of rare or exceptional but life-threatening adverse effects. Macrolides and fluoroquinoles are widely prescribed for treatment of infections and are traditionally known to cause Q-T interval prolongation and are at risk to induce Torsade de Pointes (TdP) that represent one of the most dangerous effects with these drugs. Recently, erithromycin was found to induce TdP, with relative high frequency, even if administered by oral route. A recent trial conclude that short term clarithromycin in patients with stable coronary heart disease may significantly cause higher cardiovascular mortality. Genes who are involved in congenital long-QT (cLQT) syndromes may also be involved in acquired LQT (aLQT), induced by drugs. Studies of genetic risk factors have been performed in the United States and Western Europe, suggesting that genetic mutations in genes codifing for potassium channels and involved in cLQT may be common in patients with drug-induced arrhythmias. Some studies have shown prolongation of the QT interval and blockade of the potassium channel encoded by HERG gene. HERG forms voltage-gated K channels that are associated with Mink-related peptide 1 (MIRP1), encoded by KCNE2 gene. Mutations in HERG and KCNE2 genes may be correlated with susceptibility to drug-induced arrhythmia.
Methods: Venous blood samples from 25 unrelated patients and of 40 unrelated subjects, as control, were taken after informed consent. Genomic DNA was isolated using standard procedures and PCR was carried out using specific primers that cover the complete coding regions and intron-exon boundaries. Single-strand conformation polymorphism (SSCP) analysis was performed to identify mutations in the coding regions and anomalous PCR products were sequenced.
Results: Screening for mutations in HERG and KCNE2 genes with SSCP, identified an abnormal SSCP conformer in the KCNE2 gene from DNA of one patient during treatment with erythromycin and another in HERG gene from one patient treated with clarithromycin. Sequenced analysis revealed a mutation, leading to 1-bp deletion (delA, 360) and (C1039T) respectively.
Conclusion: Individual case reports have shown that patients with macrolides-induced arrhythmia may carry sporadic mutations in HERG and KCNE2 genes. These results should be validated by further studies in a large population in order to evaluate the statistical association with polymorphisms and drug-induced arrhythmia.

P1244 Pharmacokinetic characterisation of ertapenem following subcutaneous administration in acutely ill patients

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Objective: To compare the pharmacokinetic and pharmacodynamic parameters of sequential intravenous and subcutaneous ertapenem in the plasma of surgical intensive care unit patients.
Methods: Six patients, all males, between 19 and 82 years old, with suspected hospital-acquired infection due to an ertapenem-susceptible bacteria gave their informed consent to participate. Patients with abnormal renal function (estimated creatinine clearance <30 ml/min/1.73 m^2), cardiac failure, receiving vasopressor agents or having a body mass index >30 kg/m^2 were excluded. Ertapenem 1g was administered first as a 30min intravenous infusion once a day. At steady-state, between the fourth and the seventh day after the antibiotic administration started, the pharmacokinetic study for the intravenous route was performed (13 blood samples). The day thereafter, the pharmacokinetic study for the subcutaneous route was performed after administration of ertapenem 1g as a 30 min subcutaneous infusion (12 blood samples). Blood samples were centrifuged for 10min at 4°C to separate the plasma, and ertapenem concentrations were measured using a validated LC-MS/MS assay. A 2 open-compartmental model with zero order administration was used for pharmacokinetic calculations and derived parameters were used for simulations.

Table Ia: Mean±SD pharmacokinetic parameters

	AUC (μg/mL×h)	Cmax (μg/mL)	Tmax (h)	MAT (h) au choix	$t\frac{1}{2}$ (h)
IV infusion	317±158	115±28	0.5	0.25	3.9±1.2
Sub-cut infusion	313±184	43±29*	2.7±1.1*	3.4±2.7	5.4±1.4

*$p < 0.01$.

Table Ib: Simulated plasma concentrations time over MIC in percentage according to various MIC values

	MIC = 1 μg/mL	MIC = 2 μg/mL	MIC = 4 μg/mL	MIC = 8 μg/mL
IV infusion	87±17%	74±19%	58±19%	40±16%
Sub-cut infusion	96±8%	91±13%	71±14%	47±14%

Results: All treatments were well tolerated. Mean±SD pharmacokinetic parameters are listed in Table Ia. The major observation was that ertapenem concentrations versus time profiles were shifted to the right after subcutaneous administration and time to peak (Tmax) was considerably delayed with peak concentrations (Cmax) reduced to about 1/3. However area under the curves (AUC) were similar for both administration route with a ratio of AUCsubcut/ AUCIV = 0.99±18)

and simulations indicate that time over MIC had a tendency to be longer after subcutaneous administration independently on the selected MIC value (Table Ib).
Conclusion: Subcutaneous administration seems to be an interesting alternative to intravenous infusion in acutely ill patients.

P1245 Variability in cefazolin concentrations in amniotic fluid: the relevance of gestational age

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Objectives: Cefazolin is frequently administered for prophylaxis during foetal surgical interventions, but data on amniotic drug disposition and the determinants of its variability during pregnancy are still limited. We therefore wanted to document cefazolin disposition in the amniotic fluid and the determinants of its variability.
Methods: A first dose of cefazolin (2 g iv) was administered (time interval 30 min) one or two hours before foetal surgical intervention. Amniotic fluid sampling was performed when puncture of the amniotic cavity and subsequent sampling of amniotic fluid was part of the routine clinical care without addition burden for either mother or foetus. Time of sampling (min) was registered and gestational age (GA, weeks) at surgery was recorded. Cefazolin concentrations were determined based on High Performance Liquid Chromatography methodology (1). Data were reported by median and range, spearman rank correlation and multiple regression were used to assess the impact of time of sampling and GA at surgery on the variaibility in cefazolin concentrations observed in the amniotic fluid.
Results: 57 amniotic fluid samples were collected during foetal surgical interventions. The median cefazolin amniotic concentration was 0.82 (range 0.06–3.73) mg/L. Median GA was 26 (range 17–34) weeks, median time at collection was 100 (range 20–370) minutes. There is a significant increase in cefazolin concentration with time (r = 0.41, 95% CI 0.17–0.6) and with GA (r = 0.58, 95% CI 0.37–0.73). In a multiple regression model, both variables remained independent determinants of the amniotic cefazolin concentration (r adj = 0.35)
Conclusions: There is important variability in cefazolin concentrations in amniotic fluid during pregnancy. In addition to time of sampling after intravenous maternal administration, gestational age also contributes to this variability, suggesting the impact of maturational aspects (protein binding, placental transfer, foetal renal elimination) on cefazolin disposition during pregnancy.

Reference(s)

[1] Vella-Brincat JW, et al. Br J Clin Pharmacol 2007; 63: 753–7.

P1246 Cerebrospinal fluid compartmental pharmacokinetics of amikacin in neonates

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Objectives: To describe and investigate covariate effects (leukocytosis, glycorrhachia, protein concentration) on cerebrospinal fluid (CSF) pharmacokinetics of amikacin in neonates.
Methods: CSF samples were prospectively collected from neonates in whom amikacin had been initiated before a diagnostic lumbar puncture was performed. CSF analysis (amikacin concentration, white blood count, glucose content, protein concentration) and amikacin therapeutic drug monitoring results (peak and trough concentrations) in plasma were recorded. A two compartment (central and CSF) linear disposition model was used to estimate population pharmacokinetics. The half-time (Teq) for equilibration between plasma and CSF compartments was used as a measure of blood brain barrier permeability.
Results: Based on 44 CSF amikacin concentrations and 83 plasma samples available from 43 neonates, data analysis revealed two distinct groups of neonates: those with a very short Teq (0.155 SD 0.024 h) and those with a long Teq (67.9 SD 22.5 h). A white cell count above 21 cells per mm3 in the CSF was associated with a low Teq while no relationship between Teq and CSF glucose or protein could be established.
Conclusions: There is important between individual variability in Teq following amikacin administration in neonates that is associated with CSF WBC. The impact of CSF WBC on amikacin Teq is of potential relevance for both effectiveness and ototoxicity of this drug in neonates.

P1247 Contributors to between-individual variability of amikacin clearance in neonates

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Objectives: We recently documented that size (49%), renal function (14%) and postmenstrual age (PMA 18%) are the major contributors to vancomycin clearance variability in neonates, leaving only 18% of variability unexplained. A variable slope sigmoidal model fitted best to describe the relationship between vancomycin clearance and PMA (1). A similar exercise in a large dataset of amikacin concentrations in neonates was undertaken to test applicability of the current model to others drugs, like amikacin, cleared by renal elimination.
Methods: Population pharmacokinetics were estimated (NONMEM) in a cohort of 715 neonates (PMA 24–43 weeks; weight 0.385–4.780 kg) and were based on 1 862 amikacin time-concentration points. Covariate analysis included weight, PMA, postnatal age (PNA), creatinaemia, use of inotropes or ibuprofen, positive blood culture and respiratory support. A one-compartment linear disposition model with zero order input and first order elimination was used.
Results: The population estimate for volume of distribution (V) was 32.1 L/70kg and 1.21 L/h/70kg for clearance (CL). CL increased from 0.84 L/h/70kg at 28 weeks PMA to 1.23 and 1.56 L/h/70kg by 34 and 40 weeks PMA. Overall 92% of the variability of CL was predictable. Size explained 66%, PMA 17% and renal function 9%. The use of a variable slope sigmoidal model to describe the relationship between clearance and PMA predicted an adult amikacin clearance of 3.87 L/h/70kg.
Conclusions: The explained variability of clearance and the relative contribution of the different contributors (size, PMA and renal function) is a similar magnitude with the earlier reported observations on vancomycin clearance and a variable slope sigmoidal model fitted best to predict adult amikacin clearance. These findings strongly suggest that the current model likely describes the underlying maturation of renal drug clearance. The development and subsequent validation of such models should further improve the predictability of drug clearance for drugs cleared by a similar route.
Anderson B et al. Br J Clin Pharmacol 2007;63(1):75–84

P1248 Cerebrospinal fluid penetration and pharmacodynamic exposure of meropenem prophylactically administered to neurosurgical patients

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Objectives: Meropenem is frequently used for the treatment and prophylaxis of bacterial meningitis. However, the drug's distribution within the site of action is poorly understood. This study examined the penetration and pharmacodynamic exposure of meropenem into cerebrospinal fluid (CSF).
Methods: Meropenem (0.5 g every 8 h) was prophylactically administered to six patients who underwent neurosurgery and external lumbar drainage for the management of cerebrovascular diseases. Lumbar CSF and venous blood samples were obtained at the end of the infusion (0.5 h) and 1, 1.5, 2.5, 4.5, and 8 h thereafter. Pharmacokinetic data in CSF and plasma were estimated noncompartmentally, analysed using a 3-compartment pharmacokinetic model, and used for a Monte Carlo simulation with MIC data of clinical isolates in Japan.
Results: Noncompartmental pharmacokinetic analysis demonstrated that the observed maximum concentration was 0.66 ±0.17 (mean ± SD) mg/L at 3.00±1.22 h in CSF and 29.50±6.54 mg/L at 0.5 h in plasma,

and the CSF/plasma ratio was 0.024±0.011. The area under the drug concentration-time curve (AUC 0–8 h) was 3.76±0.75 mg·h/L in CSF and 35.76±6.15 mg·h/L in plasma, and the CSF/plasma ratio was 0.109±0.035. The Monte Carlo simulation combined with compartmental pharmacokinetic modeling showed that the probabilities of achieving the bacteriostatic exposure target in CSF (30% of the time above the MIC) for 0.5 g every 8 h (0.5-h infusion) were 99.8% against a *Streptococcus pneumoniae* population (MIC50 = 0.06 mg/L; MIC90 = 0.5 mg/L) and 99.6% against a *Haemophilus influenzae* population (MIC50 = 0.12 mg/L; MIC90 = 0.5 mg/L), respectively. The prophylactic regimen of 0.5 g every 8 h was considered to provide sufficient bacteriostatic exposure in CSF for the two major meningitis pathogens.

Conclusion: These results expand our knowledge of the rate and extent of meropenem penetration into the cerebrospinal fluid and space, and provide a pharmacokinetic-pharmacodynamic rationale for design of a meropenem regimen to prevent bacterial meningitis.

P1249 Altered pharmacokinetics of linezolid in patients with severe burn injuries

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Background: Significant physiological changes occur in patients with major thermal burn injuries and result in alterations to the pharmacokinetics (PK) of many agents. These changes usually result in increased drug clearance and therefore it is necessary to increase the dosage of many antiobiotics. Today there is no data available describing the pharmacokinetics of linezolid (LZD) in burn patients and thereby whether doses need to be adjusted in these patients.

Methods: LZD PK were studied in 8 patients with severe burn injuries compared to 8 healthy volunteers matched for gender and weight. Mean (SD) demographic factors for the patients and volunteers were, age 37.0 (11.1) y vs. 37.6 (9.0) y; weight 64.0 (10.7) kg vs. 68.5 (9.4) kg and burnt area 41.0 (20.7) % vs. 0 (0) %. 600 mg LZD was administered as a 1 h infusion and both blood and urine samples were taken up to 72 h post dose and assayed by a validated HPLC method. PK parameters were determined by non-compartmental analysis.

Results: The mean (SD) PK parameters determined for LZD in the patients and healthy volunteers were: AUC0–∞ 42.5 (24.0) mg.h/L vs. 98.1 (29.2) mg.h/L, half life 2.1(1.0)h vs. 4.8(2.0)h and Vss 50.8 (16.8) L vs. 39.6 (5.7) L, respectively. The differences for AUC and T1/2 were statistically significant (P = 0.016; Wilcoxon signed rank sum test), but not the difference for Vss (P = 0.383). Mean concentrations of LZD dropped below the susceptibility breakpoint (EUCAST ⩽4 mg/L) for staphylococci after 4 h in the patients and 10 h in the volunteers.

Conclusion: The PK of LZD were significantly altered in patients with major burn injuries as compared to healthy volunteers. The drug exposure, as assessed by AUC, was reduced by approximately 50% in burn patients suggesting that a different dosage schema might be required. Today it is not clear how this possible difference should be addressed, but for a drug that has time-dependent efficacy, it may be more appropriate to increase the frequency of administration rather than the dose administered.

P1250 Bioequivalence of marketed voriconazole oral suspension and tablet formulations: results of a three-period, crossover, multiple-dose study

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Objectives: Voriconazole (VORI) is an extended-spectrum antifungal drug widely used in the treatment of opportunistic fungal infections, including invasive aspergillosis and candidaemia. An oral suspension of VORI has been developed for use in adult patients who are unable to swallow marketed tablets, and in paediatric patients. This study assessed the bioequivalence of two batches of VORI oral suspension (batches differing only in drug particle size of the in-going drug substance) relative to VORI tablet. The safety and tolerability of the two formulations were also compared.

Methods: This was a randomised, three-period, crossover, multiple-dose study, carried out in fasted healthy male subjects aged 18–45 years. In each study period, subjects received VORI tablet or suspension (batch 1 or 2) 400 mg twice daily on Day 1 followed by 200 mg twice daily for 5.5 days; study periods were separated by a wash-out period of at least 7 days. Blood samples were taken on Day 7 to assay VORI plasma concentrations at specified times up to 12 hours post-dose. The safety analysis included assessment of adverse events, clinical laboratory tests and physiological parameters.

Results: Pharmacokinetic data were obtained for 48 subjects randomised to receive VORI: suspension batch 1, n = 43; suspension batch 2, n = 44; tablet, n = 45. Statistical analysis of these data (Table) demonstrated that both VORI suspension batches were bioequivalent to the tablet formulation, as 90% CIs for suspension batch 1/tablet and suspension batch 2/tablet ratios both fell within the standard AUC_{tau} and C_{max} equivalence ranges of 80–125%. The incidence and types of adverse events were consistent with the known VORI safety profile, with similar safety profiles across the three drug formulations.

Conclusion: Both batches of VORI oral suspension were bioequivalent and similar in safety to VORI tablet. The availability of VORI oral suspension can provide further flexibility in patient care, an important component in the successful management of invasive fungal infections.

Table: Statistical analysis of pharmacokinetic parameters of voriconazole suspension and tablets on Day 7.

Parameter	Adjusted means*		Ratio or Difference†	90% CI
	Suspension batch	Tablet		
Suspension batch 1 vs tablet				
AUC_τ (ng·h/ml)	11718	11478	Ratio: 102%	(99, 106)
C_{max} (ng/ml)	2253	2166	Ratio: 104%	(98, 111)
T_{max} (h)	0.95	1.39	Difference: 0.44 h	(–0.66, –0.23)
Suspension batch 2 vs tablet				
AUC_τ (ng·h/ml)	12067	11478	Ratio: 105%	(102, 109)
C_{max} (ng/ml)	2488	2166	Ratio: 115%	(108, 122)
T_{max} (h)	0.82	1.39	Difference: 0.57 h	(–0.78, –0.36)

AUC_τ = area under the plasma concentration time curve over dosing interval τ; C_{max} = maximum observed plasma concentration; T_{max} = time to first occurrence of C_{max}.
*Adjusted means are geometric for AUC_τ and C_{max}, and arithmetic for T_{max}.
†Ratio = suspension/tablet. Difference = suspension minus tablet.

P1251 Effect of voriconazole on the pharmacokinetics of reduced doses of sirolimus

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Objectives: Sirolimus (SIR) is metabolised by CYP3A4/5, while voriconazole (VORI) inhibits CYP3A4-mediated drug clearance. Systemic exposure of SIR (with standard 2 mg dose) is significantly increased by VORI coadministration. Hence, concomitant use of VORI and SIR is contraindicated. Our aim was to determine SIR dose adjustments, when coadministered with VORI, which result in exposures similar to monotherapy.

Methods: This was an open-label, nonrandomised, parallel-group, multiple-dose, fixed-sequence, 2-period study in 30 healthy males. The effect of steady state oral VORI on the multiple-dose pharmacokinetics (PK) of 3 reduced doses of SIR (oral solution; 0.25, 0.15 and 0.10 mg/d; n = 30/group) was investigated relative to the standard dose alone in the same subjects. Serial blood samples were collected at predose and over a 24-h interval on the final day of each study period; SIR and VORI concentrations were determined by LC/MS/MS. PK were determined by noncompartmental methods. A modelling approach was used to estimate the effect of staggered VORI dosing on steady state SIR levels.

Results: The changes in PK parameters when reduced doses of SIR were coadministered with VORI are shown in the table.

Treatment		AUC_{24} (ng·h/mL)	C_{max} (ng/mL)	C_{min} (ng/mL)	$t\frac{1}{2}$ (h)
Group 1	Day 4	93.6	10.1	2.5	17.9
	Day 9	125.6	8.3	4.3	30.5
Group 2	Day 4	82.0	9.3	2.0	18.9
	Day 9	100.4	5.6	3.4	32.6
Group 3	Day 4	106.6	11.3	2.6	20.6
	Day 9	86.6	4.6	3.2	36.4

Mean PK parameters of SIR. SIR was given at standard dose on Days 1–4 and at reduced dose on Days 6–9: 0.25 mg/d (group 1), 0.15 mg/d (group 2) and 0.10 mg/d (group 3). Standard-dose VORI was given on Days 6–9.

A decrease in SIR AUC24 of 16% (90% CI: 8%-24%), relative to standard-dose SIR was observed for 0.10 mg SIR with concomitant VORI, resulting in suboptimal exposure to SIR. Conversely, AUC24 was increased by 23% (90% CI: 11%-36%) and 36% (90% CI: 21%-54%) with SIR doses of 0.15 and 0.25 mg, respectively, resulting in slightly higher exposure to SIR than with standard-dose monotherapy. Across all 3 study groups, SIR C_{max} values were lower by 19%-59%, Cmin values were higher by 9%-64% and $t\frac{1}{2}$ values were higher by 70%-77% compared with standard-dose monotherapy. SIR had no effects on VORI PK. Modelling indicated that staggering dosing of VORI and SIR is likely to decrease the magnitude of interaction.
Conclusion: It is possible to coadminister reduced doses of SIR with VORI to achieve systemic exposures similar to standard-dose SIR monotherapy. While not specifically tested in this study, our results suggest that a 90% reduction in SIR dose would maintain SIR therapeutic levels when coadministered with VORI. Our results are applicable only when both drugs are administered simultaneously orally. Further studies assessing the PK, safety and efficacy of coadministration of reduced SIR doses and VORI in transplant patients are necessary.

P1252 Population pharmacokinetic analysis of moxifloxacin in patients undergoing cardiac surgery

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Objective: A population pharmacokinetic (PPK) model for moxifloxacin (MXF) in the serum, heart tissue (right atrium and ventricle) and aortic root of patients undergoing scheduled aortic valve surgery (AVS, Ross procedure) was to be developed including an estimate of the influence of the cardiopulmonary bypass and patient characteristics on the PPK of MFX.
Methods: The PPK model was based on data from 20 patients who had undergone AVS. Patients underwent surgery at 3, 7, 10, 13 or 20 h after start of a 1 hour 400 mg MFX infusion. Data collected included MFX/serum concentration profiles and tissue samples. Demographic, renal parameters, and the AVS procedure were tested as covariates using the NONMEM software.
Results: The PPK of MFX was described by a three-compartment model (population mean [% relative standard error] volume of central compartment Vc=57 L [18%], elimination rate ke=0.17 1/h [14%], clearance CL =9.8 L/h) with a moderate inter-individual coefficient of variance of 20% for Vc and 26% for ke, about half of which was attributable to an effect of weight and sex on Vc and the effect of renal function (serum creatinine) on ke. The model implied that the MFX serum concentrations were transiently reduced by about 20% during application of the priming fluid of the cardiopulmonary bypass. The tissues behaved as small compartments directly linked to the central compartment, resulting in swift attainment of high levels of MFX in tissue. Equilibrium with serum was reached rapidly in the right atrium and right ventricle, indeed within 1 hour of the end of infusion. The aortic root tissue reached equilibrium within 2–5 hours. The latter is in line with differences in perfusion and comparable with other fast equilibrating tissues.
Conclusion: Despite the sparse sampling, a consistent model was achieved that describes the fast penetration of MFX into cardiac tissue. The PPK results support the possible use of MXF in infectious endocarditis.

P1253 Faropenem pharmacokinetics in AOM children following oral administration of faropenem medoxomil

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Objectives: Faropenem medoxomil (FM) is a novel oral prodrug antibiotic with in-vitro activity against common repiratory tract pathogens including activity against penicillin and MDR resistant *S. pneumoniae*. The objective of this dose-ranging study was to evaluate faropenem (FAR) pharmacokinetics in paediatric AOM.
Methods: In this phase II study, children with AOM (0.5–7yrs) received FM BID for 10 days. Blood was collected from 179 children at 0.5, 1, 2 and 4 hours post dose 1. In addition, a single blood sample was obtained at study Visit 2 (study Days 4–6) in 314 children over a range of time following dose administration on that day. Doses evaluated were 7.5, 15, 30 and 40 mg/kg. FAR plasma concentrations were determined using a validated LC-MS/MS assay with a lower limit of quantitation of 2.5 ng/mL. Individual subject FAR pharmacokinetic parameters on Day 1 were calculated using non-compartmental analysis.
Results: FAR plasma pharmacokinetics following oral administration of FM on Day 1 demonstrated increasing exposure with increasing dose. Median FAR Cmax values were 16.5, 26.0, 54.3, and 57.9 mg/L at doses of 7.5, 15, 30, and 40 mg/kg, respectively. Corresponding median AUC0–4 values were 20.4, 42.3, 93.0 and 112 mg*h/L, respectively. FAR plasma concentrations at Visit 2 were similar to those observed on Day 1.
Conclusions: FAR pharmacokinetic data in paediatric subjects confirmed dose-linearity of AUC, while C_{max} values, although approximately linear, trended toward less than expected increases with increasing dose. Comparison of paediatric values with those observed in adults showed an increase of 1.8-fold in CL/F. As expected with a drug that is renally eliminated, a correlation was observed between increasing AUC with decreasing CrCl. No gender or age (0.5 to 7 years) related effects were observed with FAR pharmacokinetics. Similar FAR plasma concentrations were observed following repeat dosing indicating no substantial changes in FAR pharmacokinetics over time.

P1254 The pharmacokinetics of posaconazole administered orally versus via a nasogastric tube in healthy subjects

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Objectives: The pharmacokinetics (PK) of single-dose posaconazole (POS) given either orally (PO) or via nasogastric (NG) tube were evaluated in healthy subjects.
Methods: This was an open-label, comparative, cross-over study in healthy subjects. In random sequence, subjects received POS 400 mg PO or NG (Day 1) followed by a 7-day washout and then a second dose of POS 400 mg via the alternate route on Day 8. POS was administered 5–10 minutes after completion of 8 fluid oz of Boost Plus® administered via the same route as POS in each dosing period. Blood samples for POS plasma concentration determination by LC/MS-MS method were obtained at prespecified time points over a 12-h period following POS administration. The log-transformed PK parameters from a noncompartmental analysis were statistically analysed using an ANOVA model.
Results: Fifteen subjects (male and female) completed both treatment periods. Cmax and AUC values for NG tube administration were 81%

and 76% of those observed following PO administration. The median Tmax values were the same for both routes, indicating that rates of absorption of POS is unaffected by PO and NG tube routes of administration. The mean apparent clearance (CL/F) was 12% higher for NG tube than that for the oral route of administration. It is likely that this difference is to due to differences in the amount of POS absorbed from the different administration routes. The elimination as indicated by terminal phase half-life (t1/2) of POS would be expected to be similar regardless of the route of administration. Consistent with this, the t1/2 values were similar between PO administration and NG Tube administration. PO and NG tube administration of POS 400 mg was found to be safe and well tolerated when administered to healthy adult subjects.

Table 1: Mean and % CV of POS PK parameters in healthy subjects following administration of a 400 mg dose (suspension) orally (PO) or via a NG Tube 5–10 minutes after receiving a nutritional supplement (Boost Plus®)

Parameter	NG Tube		PO	
	Mean	% CV	Mean	% CV
Cmax, ng/mL	266	45.2	324	49.7
Tmax, h	4.0[a]	2.0–6.0[b]	4.0[a]	3.0–12.0[b]
AUC(I), ng·h/mL	8402	44.8	10866	53.4
t1/2, h	23.7	30.7	21.6	38.9
CL/F, mL/h	594109	52.3	49345	54.2

[a]Median; [b]Range.

Conclusion: In healthy subjects, NG tube administration of a single dose of 400 mg of POS (suspension) appears to lead to Cmax and AUC values that are about 80% of those observed following PO administration when both were administered 5–10 minutes after 8 fluid oz of Boost Plus®.

P1255 Pivmecillinam: estimation of adequate dosage for susceptible and ESBL-producing *E. coli* by Monte Carlo PK/PD simulation

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Objectives: Pivmecillinam (PM) is widely used in the Scandinavian countries for treatment of urinary tract infections (UTI). It is highly active against non β-lactamase producing *E. coli* with MIC values around 0.125–0.5 mg/l, but also retains activity against β-lactamase producing, even many ESBL-producing, *E. coli*, where the MIC has increased to a level of 1–2 mg/l. We therefore wanted to estimate the probability of target attainment in serum for a number of dosage regimens for the two different MIC levels.

Methods: Pharmacokinetic data for mecillinam concentrations after per oral dosing of 400 mg PM (equivalent to 273 mg mecillinam) were on file at LEO Pharma. 11 measurements of serum concentration within 8 hours from intake were recorded in 17 individuals (age 26.6±7.3 years, weight 73.0 ±13.9 kg). The NPAG program was used to fit a linear compartment model (seven state variables, six PK parameters) to the data whereby mean values as well as the covariance matrix for the PK parameters were obtained. Monte Carlo simulations were then made with the compartment model assuming lognormal as well as nonparametric (empirical) frequency distributions for the PK parameters. Results were obtained on the probability of target attainment with respect to a Time>MIC of 40% as usual for most β-lactam antibiotics. Since the drug concentrations in serum are most important for treatment of renal infection, these were used as surrogate markers in this PK/PD analysis.

Results: After the parameter estimation process, the compartment model was able to reproduce the measured serum concentrations with satisfactory precision. With a MIC of 0.25 mg/l, a Time>MIC of 40% (for an unbound fraction eq. to 90% of total dose) could be reached for 95% of the simulated population on a 400 mg PM dose TID. The same population coverage (95%) required 800 mg QID if the MIC was around 1 mg/l.

Conclusion: The lower dose fits the dosage regimen presently recommended for treatment of upper UTI. The high dose is still manageable since PM has low toxicity; a total dose of 60 mg/kg is tolerated in mature humans. Based on the simulation results, a standard PM dose of 400 mg TID is recommended for upper UTI. For ESBL-producing *E. coli*, if clinical data allow, the dose should be increased to 800 mg QID.

P1256 Three-times weekly teicoplanin in the outpatient treatment of chronic infections

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Background: Several types of infections deserve prolonged treatment for months. In the setting of infections caused by multidrug resistant Gram-positive cocci, outpatient treatment is difficult to perform especially in the case of exclusive parenteral options.

Method: We enrolled in this study with teicoplanin three-time weekly patients who need to be treated with teicoplanin for prolonged time. After a loading dose of 7 mg/Kg everyday for 15 days, patients received 12 mg/Kg on Monday, Wednesday and Friday in an outpatient setting. Trough and peak concentrations were determined by fluorescence polarisation immunoassay (Seradyn, USA). Serum concentrations of teicoplanin were also determined in a control group of inpatients receiving teicoplanin (7 mg/Kg) everyday.

Results: Thirty-three patients were enrolled: 14 with vertebral osteomyelitis, 11 with infected orthopaedic implants, 5 with osteomyelitis, 3 with arterial prosthetic infections. Teicoplanin was administered empirically in 10 patients, for MRSA infections in 12 patients, for MR-CNS infections in 9 patients, for MSSA infection in 1 patient and for *Propionibacterium* spp. infection in 1 patient. The duration of treatment ranged from 60 to 360 days (median 150±84 days). Cure was obtained in 21 (63%) and improvement in 12 (37%) cases. Trough and peak serum concentrations in three-time weekly patients were 16.2±7.2 and 58.7±14.4, respectively. In the control group trough and peak serum concentrations were 18.9±13.6 and 52.2±27. Adverse effects occurred in 6 patients and caused discontinuation of treatment only in 1 patient.

Discussion: Three-time weekly teicoplanin was effective in the treatment of chronic infections. This way of administration yields the same serum levels of teicoplanin when the drug is administered everyday at standard doses, while adverse effects are rare and mild.

P1257 Value of pharmacokinetic/pharmacodynamic in dose management of ceftazidime and imipeneme in ICUs

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Objectives: the purpose of our study was to assess the value of serum assay of ceftazidime (CAZ) and imipeneme (IMI) in patients in the intensive care unit (ICU) of the Saint-Etienne University Teaching Hospital and in other ICUs in the region with regard to optimisation of treatment management.

Methods: Between 01/11/05 and 31/10/07, in patients hospitalised in ICUs, not on dialysis and undergoing treatment with CAZ given in a continuous infusion or with IMI (non-continuous), serum assay of the respective antibiotics was performed 36 hours after the start treatment using a single serum sample for CAZ and determination of trough and peak concentrations for IMI. Assays were performed using the microbiology technique with results 18 hours after sample collection. CAZ 2 g was given systematically in a bolus at the start of treatment.

Results: assay was performed in 81 and 95 patients respectively for CAZ and IMI. Mean patient age was 66 years (19 to 89 years) and mean weight was 73 kg (33 to 122 kg). The dosage was between 1 g and 6 g/24 h for CAZ and between 500 mg and 6 g/24 h for IMI. The mean serum CAZ concentration was 47.6 mg/L (7.4 to 162.3 mg/L).

Serum CAZ concentrations were as follows: 35 to 65 mg/L in 37% of patients, <35 mg/L in 43.2% and >65 mg/L in 19.8%. Infection was established in 61 patients, with 52 strains of *P. aeruginosa* detected. The serum concentration/MIC ratio was $\geqslant$5 for 84.4% of patients and >10 for 65.6% of patients. Trough concentrations of IMI were <0.5 mg/L for 14.7% of patients, between 0.5 and 2 mg/L for 43.2%, and >2 mg/L for 42.1%. The mean peak concentration of IMI was 19.9 mg/L (3 to 78 mg/L). Infection was recorded in 52 patients, including 22 *Enterobacter* spp. and 12 *P. aeruginosa*. Antibiotic dosage was adjusted respectively for CAZ and IMI in 19.8% and 28.4% of patients based on the initial assay results.
Conclusion: Our study shows that assays are needed in ICUs to confirm the efficacy of time-dependent antibiotics, to avoid treatment toxicity, to achieve efficacy as rapidly as possible and to avoid selection of resistant mutants, particularly in strains having limited susceptibility to antibiotics.

P1258 Moxifloxacin does not require dosage adjustment in patients with severe hepatic insufficiency

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Objectives: Severe bacterial infections such as spontaneous bacterial peritonitis (SBP), pneumonia, or sepsis are well-described life-limiting complications in patients with advanced liver cirrhosis. Pharmacological and microbiological characteristics make moxifloxacin an attractive option in the treatment of such infections. On the other hand, since moxifloxacin is predominantly metabolised by the liver, a drug accumulation theoretically can not be excluded in patients with advanced liver insufficiency. Due to a lack of pharmacokinetic data the therapeutical use of moxifloxacin is currently not recommended in this patient population.
Patients and Methods: In a single-centre, prospective, open-label study, 9 adult cirrhosis patients with severe liver insufficiency (Child–Pugh class C) and an infection (pneumonia or SBP) were treated with 400 mg moxifloxacin infusion once a day. On days 1 and 3 drug concentrations in serum and ascites were determined before and at different time points up to 24 h after medication with a validated HPLC method.
Results: On day 1, serum concentrations of moxifloxacin decreased from a geometric mean of 3.60 mg/L at 1 h to 0.62 mg/L at 24 h. On day 3, serum peak and trough levels showed only a moderate accumulation. The AUC values were slightly, but not statistically significant, increased on day 3. Calculations of t1/2, MRT, CLtot, and Vss also revealed no significant differences between days 1 and 3. Mean concentrations of moxifloxacin in ascitic fluid were 1.47 mg/L at 3.0 h and 1.39 mg/L at 6.0 h on day 1, and 2.07 mg/L at 3.0 h and 1.88 mg/L at 6.0 h on day 3. There were no differences in mean ascites/serum ratios between days 1 and 3.
Conclusions: PK parameters of intravenous moxifloxacin in patients with advanced liver cirrhosis differed only marginally from those from healthy control groups given in the literature. After multiple dosing only a moderate drug accumulation was seen. Therefore, we conclude that a dose adjustment is not necessary in this patient group. In addition to this, ascitic fluid reached bactericidal levels of moxifloxacin for common bacteria found in spontaneous bacterial peritonitis.

P1259 Penetration of moxifloxacin into the tissue of heart and aortic root of patients

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Objective: Moxifloxacin is clinically used in respiratory tract and skin and soft tissue infections. The aim of the present study was to provide pharmacokinetic data for the possible use of moxifloxacin in infectious endocarditis.
Methods: 20 patients (12m/8f) with age (median, range) of 53, 23–64 years, weight 84, 61–106 kg, height 175, 156–195 cm, BMI 27.1, 20.7–48.8 kg/m2) undergoing scheduled aortic valve surgery (Ross procedure) were enrolled into the study. During surgery, tissue specimens were sampled in 4 patients each 3, 7, 10, 13 and 20 hours after start of a 1hour infusion of moxifloxacin 400 mg. Blood was sampled in order to compare tissue and concomitant serum concentrations. Moxifloxacin was determined by HPLC and fluorimetric detection in serum and tissue homogenate.
Results: The serum concentrations (median) of moxifloxacin were 1.9 mg/L after 3 hours and 0.54 mg/L after 20 hours. Moxifloxacin penetrated fast into different structural compartments of the heart. The peak concentrations in atrium and ventricle were measured already 3 hours after the start of the 1hour infusion, the penetration into the aortic root was somewhat slower. The global tissue concentration/serum ratio was 3.4–6.8 in ventricle, 2.6–4.8 in atrium and 2.2–2.8 in aortic root (see Table).

Tissue concentrations (median, range), concomitant serum concentrations and median tissue to serum ratio (a/s right atrium/serum, v/s right ventricle/serum, ar/s aortic root/serum) in patients (n = 4/group) following a 1 hour infusion of moxifloxacin 400 mg

t (h)	serum (mg/L)	atrium (μg/g)	ventricle (μg/g)	aortic root (μg/g)	a/s	v/s	ar/s
3	1.90, 1.41–2.39	8.93, 3.25–15.5	12.8, 5.53–36.5	4.66, 2.41–6.93	4.8	6.8	2.5
7	1.05, 1.03–1.44	3.14, 2.91–4.59	5.90, 5.46–6.62	2.50, 2.41–5.13	3.0	5.4	2.4
10	1.48, 0.99–1.60	2.77, 2.08–3.47	5.00, 3.50–6.69	2.37, 2.09–3.52	3.0	5.4	2.8
13	0.88, 0.53–1.44	2.07, 1.33–4.30	3.43, 1.60–5.03	1.96, 0.97–3.02	2.7	3.4	2.2
20	0.54, 0.48–0.71	1.39, 1.12–2.00	2.34, 2.14–2.63	1.42, 0.96–1.85	2.6	4.2	2.3

Conclusion: From the pharmacokinetic point of view, moxifloxacin seems to be adequate for the treatment of infectious endocarditis, caused by susceptible pathogens.

P1260 Comparison of the activity of ciprofloxacin vs. levofloxacin against *Pseudomonas aeruginosa* by the continous antibiotic gradient method: a pharmacodynamic approach in respiratory infection

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Objectives: The continous antibiotic gradient method allows to assess the risk of resistant mutant selection. In this study, the activity of levofloxacin (LVX) was compared to that of ciprofloxacin (CIP) against 50 clinical isolates of *Pseudomonas aeruginosa*. A pharmacodynamic approach was performed in order to evaluate this risk in the respiratory tract.
Methods: The antibiotic gradients were prepared in Mueller Hinton agar plates by applying filter paper strips containing different concentrations of antibiotic at regular intervals at the surface of the plate. Six different inocula, from 10–5 to 10–11 CFU/ml, were tested for each strain. Two parameters, the length of homogenous growth and the length to the most resistant colonies were measured and converted into antibiotic concentrations: boundary concentration (BC) and no growth concentration (NGC) respectively. The pharmacodynamic approach was perfomed by dividing the antibiotic concentrations observed in the epithelial lining fluid (ELF) by the NGC50. The following concentrations in ELF observed at 4 hours were used: 22 mg/L with levofloxacin 750 mg once daily or 500 mg bid and 1.9 mg/L with ciprofloxacin 500 mg bid respectively.
Results: The evolution of mutant selection was parallel between CIP and LVX: NGC50 increased by 3–5 fold from a weak to an heavy inoculum. This value refered to the observed in situ antibiotic concentrations showed a clear superiority of levofloxacin vs. ciprofloxacin, confirmed by the individual analysis of each strain.

	Inoculum				
Dilution factor	10^{-4}	10^{-3}	10^{-2}	10^{-1}	Pure
NGC50 CIP	0.84	1.03	1.76	2.02	2.31
NGC50 LVX	1.5	2.9	5.1	6.8	9
CP/NGC50 CIP	2.3	1.8	1.1	0.9	0.8
CP/NGC50 LVX	14.7	7.7	4.3	3.2	2.4

Conclusion: the in vitro selection of *P. aeruginosa* resistant mutants is similar between levofloxacin and ciprofloxacin. The emergence of resistant mutants was observed with the same frequency and the MIC increase is close. Taking into account the lung concentrations, levofloxacin shows a clear superiority over the ciprofloxacin.

P1261 Analyses supporting phase 2 clinical trial dose selection for zabofloxacin in community-acquired pneumonia

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Objective: To conduct analyses in support of dose selection for zabofloxacin in Phase 2 community acquired pneumonia (CAP) studies. Phase 2 clinical trials will be initiated for zabofloxacin, a new antimicrobial agent belonging to the fluoroquinolone class. Zabofloxacin has high in vitro activity against pathogens associated with CAP/respiratory tract infections, including penicillin- and levofloxacin-non susceptible strains of *Streptococcus pneumoniae*.
Methods: Monte Carlo simulation (5,000 iterations) using Phase 1 pharmacokinetic (PK), protein binding, and non-clinical PK-PD data were utilised to determine the probability of attaining free-drug AUC:MIC target thresholds for daily doses of zabofloxacin ranging from 50 to 800 mg. PK data were derived from a randomised, placebo-controlled, ascending multiple-dose (dose range 200–800 mg daily orally for 7 days) study in 24 healthy volunteers. Zabofloxacin clearance (CL/F) was linear over the dose range studied, with a mean (SD) of 36.3 (12.8) L/hour. A point estimate of zabofloxacin binding to serum proteins of 77%, as estimated by ultra-filtration methodology, was used in the simulations. Free-drug AUC:MIC was calculated as the product of f, where f is the fraction unbound, and dose/CL/F. A log normal distribution for CL/F was assumed. Resultant free-drug AUC values were divided by fixed clinically relevant MIC values for zabofloxacin against pneumococci ranging from 0.008 to 0.25 mg/L (MIC50/90/99 for zabofloxacin against *S. pneumoniae*: 0.015/0.03/0.06 mg/L). The free-drug AUC:MIC target threshold evaluated in these analyses was 30, which was associated with complete eradication of *S. pneumoniae* from the lungs of immuno-competent mice (inoculated with 106 CFU) after treatment with zabofloxacin.
Results: The probabilities of PK-PD target attainment are presented in the Table.

	MIC Range (mg/L)					
Daily dose (mg)	0.008	0.015	0.03	0.06	0.125	0.25
50	0.884	0.201	0	0	0	0
100	0.999	0.876	0.195	0	0	0
200	1.0	0.998	0.880	0.190	0	0
300	1.0	1.0	0.991	0.626	0.045	0
600	1.0	1.0	1.0	1.0	0.579	0.034
800	1.0	1.0	1.0	1.0	0.852	0.167

Note that for daily doses of 300 mg or greater, the probability of PK-PD target attainment approaches 1.0 for MIC values as high as 0.03 mg/L, the MIC90 for zabofloxacin against *S. pneumoniae*.
Conclusion: These analyses support a 300 mg (or greater) once-daily dose-regimen for zabofloxacin for the treatment of CAP associated with *S. pneumoniae*.

P1262 Treatment optimisation using vancomycin on the critical patient

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Introduction: It is more difficult to optimise antibiotic serum levels in critical patients (P). The aim of this study was to assess the therapeutic levels (20–30 μg/ml) of vancomycin (V′) on septic critical P on continuous intravenous infusion.
Materials and Methods: The study was carried out on 175 consecutive treatments in P with severe sepsis or septic shock, in a multipurpose ICU (20 beds) over 30 months. P were divided in to different categories according to degree of renal failure (RF) based on the RIFLE classification (glomeular filtration rate <60 ml/m); correlation of demographic characteristics and renal function with serum levels; evaluation of the population with and without sufficient levels of V′. Statistics: t-student test, Mann-Whitney and Pearson coefficient trending to $p < 0.05$.
Results: Average age 55.4 yrs, 137 men (78%), average APACHE II and SAPS II of 17 and 41.9 respectively. The average doses of vancomycin were 15.2 mg/kg for initial loading dose and 29.2 mg/kg on day 1 infusion. Average V′ levels for day 1, 2 and 3 were 19.2, 22.1 and 23.9 μg/ml with higher levels in women, independent of renal clearance, total body surface area and the dose of vancomycin measured in the first 24 hours: $p = 0.02$ for the 1st and 2nd day (14/18.3; 16.4/22.4). Over the total treatment period (1274 days) the V′ levels were above 20 μg/ml for 66% of the period and above 25 μg/ml for 40%. RF occurred in 31 P (17%) of which 12 (39%) had normal creatinine serum levels. In 71 P with V′1 > 20 (37%), RF occurred in 25 P (35%). The V′1, 2 and 3 were higher in the group with RF ($p < 0.05$) compared to the rest (17/27, 21/29, 23/30). The correlations between V′1, clearance and age were moderate ($r = -0.6$, $p < 0.01$, $r = 0.44$, $p < 0.01$), in the group without RF. Supratherapeutic levels (V > 30) occurred in 14 P (8.3%) of which 12 (86%) had RF and 36% (6 P) had normal renal function.
Conclusions: Critical Septic P without RF showed sub-optimum levels of V′1 and V′2 (<20) in a high percentage of cases (67% and 49% respectively). Women showed higher levels of V′, and V′varied proportionally with age. Toxic levels occurred mainly in P with RF. For P without RF, doses of 15 mg /kg (loading) and 30 mg /kg/day (maintenance) seem to be insufficient on day 1. In critical patients, RF is underdiagnosed, as serum creatinine shows low sensitivity for diagnosis, and so daily clearance should be used to adjust the initial dose of antibiotics with preferential renal excretion

P1263 Administartion of probiotics decrease the risk of sepsis by bloodstream infections in patients with multiple injuries

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Background: A recent randomised clinical trial of our group (Kotzampassi et al. World J Surg 2006) has disclosed considerable reduction of the infective sequelae after oral administration of Symbiotic FORTE, the latter being a preparation of Pediococcus pentoseceus 5–33:3, *Leuconostoc mesenteroides* 32–77:1, *Leuconostoc paracasei* ssp 19, and *Leuconostoc plantarum*. The effect of treatment on microbiology of the study population has not been published and it is presented herein
Methods: Patients were remaining intubated and they were administered either placebo or probiotics for 15 days after admission. Upon presentation of signs of sepsis, a complete diagnostic work-out was done comprising cultures of blood, urine and tracheobroncial secretions (TBS).
Results: Thirteen patients of the placebo group developed bloodstream infection (36.1%) compared to seven patients of patients treated with SynbioticFORTE (19.4%, LR: 16.96, p: 0.009). From the 13 placebo-treated patients with bloodstream infection all developed signs of sepsis as opposed to the remaining 23 patients without bloodstream infection where only 11 developed sepsis (OR: 2.182, p: 0.002). From the seven SynbioticFORTE-treated patients with bloodstream infection five developed signs of sepsis as opposed to the remaining 29 patients without bloodstream infection where 12 developed sepsis (OR: 1.268, p: 0.219). The time to progression to primary bacteraemia was longer among patients treated with SynbioticFORTE compared to placebo (p: 0.0237 between groups). Twelve (33.3%) and five (13.9%) placebo-treated and probiotic-treated patients respectively developed ventilator-associated pneumonia with *Acinetobacter baumannii* as a bacterial cause (p: 0.047 between groups).

Conclusions: Probiotics contained in the oral formula SynbioticFORTE decrease significantly the risk for sepsis by bloodstream infections and the occurrence of VAP by *A. baumannii*. An effect on bacterial translocation may be proposed as a probable mechanism of action.

P1264 Effect of gastric pH, dosing regimen and prandial state, food and meal timing relative to dose, and gastro-intestinal motility on absorption and pharmacokinetics of the antifungal posaconazole

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Objectives: Absorption of posaconazole (POS) can be enhanced if POS suspension is taken with food or a high-fat meal; in some patients, this may not be feasible. Factors with a potential to affect POS absorption were analysed in a four-part, open-label, randomised, crossover study in healthy subjects.

Methods: Part 1 (gastric pH, n = 32): treatments were POS 400 mg single dose (SD) vs POS 400 mg SD + 12 oz ginger ale (acidic beverage) vs esomeprazole (proton pump inhibitor [PPI]) 40 mg QD, 3 days + POS 400 mg SD (Day 3) vs PPI 40 mg QD, 3 days + POS 400 mg SD + 12 oz ginger ale (Day 3). Part 2 (dosing + prandial state, n = 32): treatments (7 days each) were POS 400 mg BID + Boost (nutritional supplement) vs POS 400 mg BID while fasting vs POS 200 mg QID + Boost vs POS 200 mg QID while fasting. Part 3 (food/meal timing, n = 32): treatments were POS 400 mg SD while fasting vs before vs during vs after high-fat meal. Part 4 (gastric motility, n = 33): treatments were POS 400 mg SD + Boost vs metoclopramide (prokinetic) 10 mg TID, 2 days + POS 400 mg SD + Boost (Day 2) vs POS 400 mg SD + Boost + loperamide (anti-kinetic) 4 mg SD. Blood collection for analysis was up to 168 h post-POS dose. Log-transformed data were analysed with ANOVA.

Table: Mean (%CV) POS PK Parameters

Treatment Comparison	Cmax, ng/mL	AUC, h·ng/mL[a]
Part 1: Effect of Gastric pH (n = 12)		
POS 400 mg SD	151 (58)	5930 (50)
POS 400 mg SD + Ginger Ale	286 (55)	9890 (48)
POS 400 mg SD + PPI	76.7 (37)	4000 (39)
POS 400 mg SD + PPI + Ginger Ale	93.3 (34)	4510 (30)
Part 2: Effect of Dosing Regimen [BID/QID] and Prandial State (n = 12)		
POS 400 mg BID Alone (7 days)[b]	982 (62)	52300 (89)
POS 400 mg BID + Boost (7 days)[b]	1590 (61)	80600 (73)[c]
POS 200 mg QID Alone (7 days)	2300 (44)	132000 (54)
POS 200 mg QID + Boost (7 days)[b]	2160 (44)	112000 (49)
Part 3: Effect of Food and Meal Timing (n = 12)		
400 mg SD Fasted	181 (106)	4540 (42)
400 mg SD Before Meal	274 (70)	10300 (66)
400 mg SD During Meal	555 (44)	21300 (48)
400 mg SD After Meal	544 (50)	21300 (48)
Part 4: Effect of Gastric Motility		
POS 400 mg SD Alone (n = 12)	303 (40)	9070 (37)[d]
POS 400 mg SD + Metoclopramide (n = 13)	232 (32)	7960 (45)[e]
POS 400 mg SD + Loperamide (n = 12)	294 (42)	11100 (52)[f]

[a]AUC to infinity for parts 1, 3, and 4; AUC to final measurable sampling time for part 2.
[b]n = 11; no concentration data for 1 subject.
[c]n = 10; no concentration data for 1 subject; missing pre-dose concentration for 1 subject.
[d]n = 9; PK parameters for 3 subjects were excluded due to extrapolated area >25% of total AUC.
[e]n = 12; PK parameters for 1 subject were excluded due to extrapolated area >25% of total AUC.
[f]n = 11; PK parameters for 1 subject were excluded due to extrapolated area >25% of total AUC.

Results: Part 1: vs POS 400 mg SD (fasting), ginger ale increased POS Cmax and AUC by 92% and 70%; higher gastric pH (via PPI) decreased POS Cmax and AUC by 46% and 32% (Table). Part 2: vs POS 400 mg BID for 7 days (fasting), POS 400 mg BID + Boost increased POS Cmax and AUC by 65% and 66%; POS 200 mg QID plus and minus Boost increased Cmax and AUC by 137% and 157% and by 136% and 161%, respectively. Part 3: vs POS 400 mg SD (fasting), POS 400 mg administered before a high-fat meal increased Cmax and AUC by 96% and 111%; administration during and after a high-fat meal increased Cmax and AUC by 339% and 382% and by 333% and 387%. Part 4: vs POS 400 mg SD + Boost, increased gastric motility (via metoclopramide) decreased Cmax and AUC by 21% and 19%, and reduced gastric motility (via loperamide) decreased Cmax by 3% and increased AUC by 11%.

Conclusion: Posaconazole absorption is substantially affected by gastric pH, prandial state, and dose administration timing. A hierarchy of strategies, including administration with/after high-fat meal, in divided doses up to QID, with a nutritional supplement, with acidic beverages, or without PPIs can maximise POS exposure if patients are likely to have decreased absorption.

P1265 The safety, tolerability, and pharmacokinetics of the antifungal posaconazole in hepatic impairment

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Objectives: To compare the pharmacokinetics (PK) of posaconazole (POS) in subjects with different degrees of hepatic impairment and in healthy subjects and to determine the safety and tolerability of a single POS dose in subjects with different degrees of hepatic impairment.

Methods: This was an open-label, parallel-group study. Thirty-six subjects were to be enrolled: up to 18 subjects with various degrees of chronic liver disease (up to 6 subjects in these categories: mild, moderate, and severe hepatic impairment) and up to 18 healthy volunteers with normal hepatic function matched with hepatically impaired subjects for race, age, height, weight, and gender. Each subject received a 400-mg single dose of POS in the morning, after a standardised high-fat breakfast. Blood samples to determine POS plasma concentrations were collected pre-dose and up to 144 h post-dose. Additional samples were to be drawn at the outpatient visits on Day 10 (approximately 216 h), Day 14 (approximately 312 h), Day 21 (approximately 480 h), and Day 28 (approximately 648 h). Samples were analysed by an LC/MS-MS method. Log-transformed PK data were analysed with an ANOVA model.

Table: Mean (%CV) POS PK Parameters

Group	Tmax[a] (h)	Cmax (ng/mL)	AUC(tf) (h·ng/mL)	AUC(I) (h·ng/mL)	Vd/F (L)	CL/F (L/h)	t1/2 (h)
Normal vs. Mild							
Normal (n = 6)	5.00 (5–6)	689 (30)	22900 (17)	23400[b] (20)	671[b] (14)	17.7[b] (20)	26.9[b] (19)
Mild (n = 6)	6.5 (5–12)	694 (41)	32800 (37)	31700[b] (40)	754[b] (33)	14.4[b] (40)	38.5[b] (28)
Normal vs. Moderate							
Normal (n = 6)	5.50 (4–8)	517 (80)	19600 (66)	22300[b] (59)	866[b] (45)	25.1[b] (67)	26.5[b] (22)
Moderate (n = 6)	5.00 (4–6)	724 (15)	24900 (23)	25700[b] (24)	621[b] (23)	16.1[b] (19)	27.3[b] (24)
Normal vs. Severe							
Normal (n = 6)	5.00 (5–6)	608 (35)	19600 (38)	18700[b] (40)	1040[b] (60)	26.0[b] (58)	27.6[b] (20)
Severe (n = 6)	9 (6–24)	403 (31)	23700 (38)	24400 (37)	1058 (39)	18.6 (41)	43.1 (43)
Normal (pooled)							
(n = 18)	5.00 (4–8)	605 (47)	20700 (41)	21500[c] (41)	857[c] (49)	22.9[c] (56)	27.0[c] (19)
Mild, Moderate, and Severe (pooled)							
(n = 18)	6.00 (4–24)	607 (38)	27100 (36)	27100[d] (35)	827[d] (41)	16.5[d] (35)	36.7[d] (39)

Note: PK Parameters of AUC(I), CL/F, Vd/F and t1/2 for 4 subjects (1 matched to moderate hepatically impaired subject, 1 matched to a severely impaired subject, and 2 matched to moderately impaired subjects) were excluded because t1/2 could not be determined.
[a]Median (range). [b]n = 5. [c]n = 15, [d]n = 16.

Results: Mean POS Cmax was greater in subjects with moderate hepatic impairment than in healthy subjects (724 vs 517 ng/mL;

Table) but was decreased in subjects with severe hepatic impairment. Mean POS exposure, or AUC(tf), was higher in subjects with hepatic impairment (32,800 h.ng/mL [mild], 24,900 h.ng/mL [moderate], 23,700 h.ng/mL [severe]) than in healthy subjects (20,700 h.ng/mL, pooled). Median Tmax observed in subjects with normal hepatic function was approximately 5 h and was delayed to 6.5 and 9 h for subjects with mild and severe hepatic impairment, respectively. Similarly, the elimination half-life (t1/2) was prolonged to approximately 39 h and approximately 43 h in subjects with mild and severe hepatic impairment, respectively. The increase in t1/2 did not significantly affect AUC compared with that in healthy subjects, especially for subjects with severe hepatic impairment. The single 400-mg POS dose was safe and well tolerated when administered to hepatically impaired and healthy subjects. No deaths or serious adverse events occurred.
Conclusion: POS exposure in patients with hepatic impairment, regardless of degree of impairment, shows considerable overlap with that in healthy subjects. Thus, these data suggest that dose adjustment of POS in patients with hepatic impairment is not necessary.

P1266 Monte Carlo simulation of ceftaroline for pharmacokinetic/pharmacodynamic target attainment and dose selection

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Objectives: Ceftaroline (CPT) is a novel, parenteral, broad-spectrum cephalosporin. CPT exhibits excellent bactericidal activity against Gram-positive organisms, including MRSA and MDRSP, as well as common Gram-negative pathogens. The objective of this study was to determine the PK/PD probability of target attainment (PTA) by Monte Carlo (MC) simulation to guide appropriate dose selection for Phase 3 studies.
Methods: MC simulation of CPT concentrations was conducted using the S-Plus program in an advanced population PK model derived from Phase 1 (a single-/multiple-dose study in healthy subjects and a study in subjects with renal impairment) and Phase 2 data (a study in patients with complicated skin and skin structure infections [cSSSI]). CPT concentrations were simulated for 4000 subjects with normal renal function (creatinine clearance >80 mL/min) at a dose of 600 mg CPT q12 h IV administered over 1 h. Simulations were also performed for a 3-h infusion (same dose) to provide sustained plasma concentrations to achieve a high PTA. Accounting for variations anticipated in an actual patient population, the analysis assumed a 21% coefficient of variation (between-subject variability of clearance) derived from a population PK model that includes patients with cSSSI. The percentage of time (%T) of the dosing interval during which serum free drug concentration exceeded the minimum inhibitory concentration (MIC) was estimated for MIC values of 0.25, 0.5, 1, 2, 4, and 8 μg/mL. The %T>MIC was determined based on a free drug concentration of 80%.
Results: MC simulations of 600 mg CPT q12 h administered over 1 h demonstrated a mean %T>MIC of 51% (MIC=2 μg/mL). The simulated mean %T>MICs with the 3-h infusion were 59% (MIC=2 μg/mL) and 41% (MIC=4 μg/mL), respectively, as shown below.

Target MIC (μg/mL)	% T>MIC			
	1-h infusion		3-h infusion	
	Mean	LL 90% CI	Mean	LL 90% CI
0.25	98	86	99	95
0.5	89	67	94	77
1	71	51	78	60
2	51	38	59	46
4	34	25	41	31
8	18	11	20	7

CI = confidence interval; LL = lower limit.

Conclusions: Based on MC simulations in subjects with normal renal function, 600 mg CPT q12 h administered as either a 1-h or 3-h infusion will achieve a high PTA to be able to eradicate target pathogens at an MIC of 2 μg/mL. A 3-h infusion provides a modest increase in the %T>MIC and allows good coverage for the target of 4 μg/mL. Given relevant pathogen susceptibility profiles, epidemiological data, and animal PK/PD model data, both of these proposed dosing regimens will achieve a high PTA for target pathogens associated with relevant indications.

Antibiotic usage and abusage: the problems

P1267 Determinants of antibiotic use in surgical wards and in intensive care units in southwestern France

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Objective: To study factors associated with antibiotic (AB) consumption in surgical wards and in intensive care units (ICU) and their relevance for benchmarking, in the context of recent French guidelines promoting reporting of AB use to health authorities.
Method: Data were retrospectively recorded for the whole years 2002 and 2005 from a convenience sample (51 and 55 surgical wards with 1,105,232 and 991,506 PD, 35 and 31 ICU with 139,102 and 139,976 PD): consumption of AB for systemic use (defined daily doses per 1,000 patient-days: DDD/1,000 PD and per 100 admissions: DDD/100 AD), number of central-line per 1,000 PD (2005) and hospital characteristics (administrative type, length of stay (LOS), number of beds: total and for ICU and surgical wards). Additional data were obtained from nosocomial infection surveys for 13 ICU and 23 surgical wards. AB were classified according WHO recommendations (ATC-DDD system, 2005). Multiple linear regression was performed in a backward stepwise approach.
Results: Median AB consumption, expressed in DDD/1,000 PD and DDD/100 AD was 1186 and 621 in 2002 and 1416 and 932 in 2005 in ICU; 477 and 268 in 2002 and 505 and 237 in 2005 in surgical units. Administrative type of hospital was associated with total AB use in both areas with highest consumption in public hospitals. Pattern of AB use was associated with LOS in ICU: use of penicillins with betalactamase inhibitors (J01CR) was lower in ICU with higher LOS, use of glycopeptides (J01XA) was higher. Total AB use in ICU was linked with number of central-line per 1,000 PD in 2005. Surgical units in public and private hospitals had different patterns of consumption, with more first and second generation cephalosporins in private and more J01CR in public hospitals in 2005. No relationship was found between patient characteristics and AB use for the 23 surgical units providing data on patient case-mix.
Conclusion: Specific data on risk factors in ICU or surgery were difficult to collect and did not seem to be more linked with AB use than readily available characteristics such as type of hospital, LOS, number of beds and of admissions for each area, number of central-line. Therefore, these factors seem relevant as surrogate to reflect patients case-mix, and should be used for stratification when comparing AB consumption from various hospitals.

P1268 Predictors of piperacillin-resistant *Pseudomonas aeruginosa* among patients with respiratory tract infections: the predictive value of varying exposure definitions and the risk of multiple prior antibiotic exposures

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Objectives: The primary objective was to determine if different prior antibiotic exposure (PAX) thresholds affected the likelihood of piperacillin-resistance (PR) among patients with *Pseudomonas aeruginosa* (PA) respiratory tract infections. The secondary objective was to examine the relationship between number of PAXs and PR-PA within the different PAX thresholds.
Methods: Retrospective cross-sectional study of patients with respiratory *P. aeruginosa* clinical cultures between 2001 and 2005. Demographics, co-morbid conditions, microbiology, and PAX data

were collected. Each of the following represents a unique PAX: piperacillin-tazobactam, cefepime, carbapenem, fluoroquinolone, and aminoglycoside. Two analyses were performed. In the first, the following PAX thresholds were examined: >24, >48 and >72 hours. Classification and Regression Tree (CART) analysis was also used to identify the PAX threshold for each drug class associated with an increased risk of PR-PA. Within each PAX threshold, log-binomial regression model was used to identify independent predictors of PR-PA. In the second analysis, patients were categorised by number of PAX (0, 1, 2 or >3) within each PAX threshold. Similar to the first analysis, log-binomial regression model was used to identify predictors of PR-PA within each PAX threshold.
Results: In the first analysis, prior piperacillin exposure was associated with PR-PA for each PAX threshold evaluated. The association between PR-PA and the other unique PAX varied according to the PAX threshold examined; more unique PAX were associated with PR-PA as the duration of PAX threshold increased in duration. In CART, carbapenems had the shortest duration of PAX associated with PR-PA (3 days), followed by fluoroquinolones (4 days), aminoglycosides (5 days), cefepime (9 days) and piperacillin/tazobactam (11 days). For the CART-derived PAX threshold log-binomial analysis, all classes were found to be associated with PR-PA, with the exception of carbapenem. In the second model, when the unique PAX were replaced by number of PAX, the number of PAX was independently associated with an increased risk of PR-PA at each threshold examined (table 1).

Log-binomial regression analysis of multiple prior antibiotic exposures associated with PR-PA

Covariate	Prevalence Ratio	95% CI	P-value
⩾24 hours			
Number of prior exposures	1.62	(1.31–2.00)	<0.001
⩾48 hours			
Number of prior exposures	1.56	(1.27–1.93)	<0.001
⩾72 hours			
Number of prior exposures	1.77	(1.42–2.20)	<0.001
CART breakpoints			
Number of prior exposures	2.17	(1.67–2.82)	<0.001

Conclusion: The results indicate that the antibiotic classes that predict PR-PA differ depending on the threshold of PAX utilised. The results also demonstrated that number of PAX, irrespective of class, was a more consistent and better predictor of PR-PA than unique PAXs.

P1269 Are we still using too many antibiotics for acute bronchitis?

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Background: Acute bronchitis is a lower respiratory tract infection that causes reversible bronchial inflammation. In up to 95 percent of cases, the cause is viral. While antibiotics are often prescribed for patients with acute bronchitis, little evidence shows that these agents provide significant symptomatic relief or shorten the course of the illness and may in fact lead to the emergence of resistant organisms. We undertook a study to evaluate the consumption of antibiotics for acute bronchitis in British Columbia (BC), Canada.
Methods: We obtained data from the BC PharmaNet database on all outpatient oral antibiotic prescriptions from 1996 to 2005. Prescriptions were expressed as their defined daily dose (DDD) per 1,000 inhabitants according to the 2006 World Health Organisation Anatomical Therapeutic Chemical system. This data was linked to the physician billing system to determine the reason for the antibiotic prescription. Overall and class-specific rates of consumption were described by year for treatment of acute bronchitis.
Results: In 1996, the BC consumption rate for all antibiotics used for acute bronchitis was 0.98 DDD per 1000 inhabitant-days. This decreased to a low of 0.63 DDD per 1000 inhabitant-days in 2002 but since then has increased such that in 2005, the rate was 0.81 DDD per 1000 inhabitant-days. Increases in fluoroquinolone use, specifically moxifloxacin (37-fold from year 2000 to year 2005), clarithromycin (3-fold) and azithromycin (7-fold) were seen while use of tetracyclines and B lactams declined by approximately 50%.
Conclusions: Physicians continue to prescribe antibiotics for acute bronchitis and we need to implement a multi-pronged approach within the province to change prescribing.

P1270 Antibiotic usage in Queensland public hospitals

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Objectives: In Queensland Australia, all the public hospital pharmacies use a common database for drug transactions. The aim was to regularly extract data from this database in order to obtain information about antibiotic consumption across Queensland Health facilities over time. This information will then be distributed back to pharmacists, infectious diseases physicians and microbiologists in order to inform formulary restrictions and influence clinical practice change in relation to antibiotic usage at the local hospital level.
Methods: Data of all antimicrobials transactions in the pharmacy database were extracted into a custom built application. Antibacterial agents were categorised using the Anatomical Therapeutic Chemical (ATC) classification index with 2007 defined daily doses (DDD). Antibiotic use was measured using DDD per 1,000 patient days (DDD/1000 PD). Data was stratified into intensive care (ICU) based on classification as a level 2 or 3 unit, and non ICU. Outpatient use was excluded as was data from the large tertiary paediatric facility and where it was possible to identify paediatric usage in the other facilities this data was also excluded.
Results: Total antibiotic consumption for Queensland public hospitals in 2006 was 946 DDD/1000 PD and in ICUs it was 657 DDD/PD. The β-lactam antibacterials, penicillin (ATC class J01C) accounted for just over half the total usage (481 DDD/1000PD) but only a third of the ICU usage (221 DDD/1000PD). The other β-lactam antibacterials (ATC class J01D) constituted 24% percent of the usage in ICU (156 DDD/1000PD) and 18% of the overall consumption (173 DDD/1000PD). Macrolides, lincosomides and streptogramins (ATC class J01F) accounted for 16% of the ICU antibiotic usage (107 DDD/1000PD) but only 9% of the total usage (89 DDD/1000PD).
Conclusion: Antibiotic usage in Queensland public hospitals is comparable with that reported nationally and in other Australian states. This consumption however, is higher than that reported in countries such as Denmark, Netherlands & Sweden. Regular and timely feedback of these antibiotic usage rates to those who have influence and control over prescribing and formulary restrictions within the public hospital system should assist to improve prescribing practices.

P1271 Trends in antibiotic consumption over an 8-year period in a general hospital of Athens, Greece

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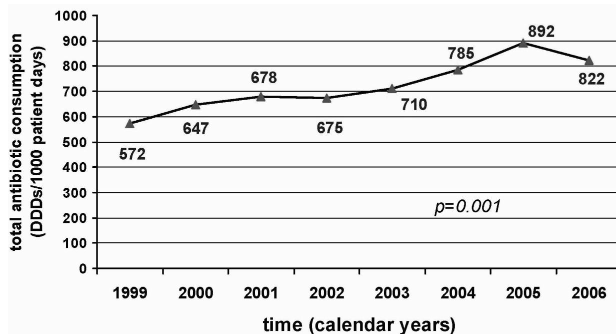
Objective: The aim of the present study was to record the trends in antibiotic consumption in our 300-bed general hospital over an 8 year period.
Methods: Antibiotic consumption for the period 1999 to 2006 was studied retrospectively using data from the pharmacy computer. Antibiotic use was calculated in DDDs per 1000 patient days (ABC Calc 3.0). We used linear regression for statistical analysis (SPSS 11.5).
Results: Antibiotic consumption was 572, 647, 678, 675, 710, 785, 892 and 822 DDDs/1000 patient days (years 1999, 2000, 2001, 2002, 2003, 2004, 2005 and 2006 respectively) and the trend was significant ($p=0.001$). Concerning consumption of major classes of antibiotics, only penicillins had a decrease (from 83.4 to 55.6 DDDs per 1000 patient days). The consumption of other classes of antibiotics had

the following trend over time: cephalosporins/aztreonam from 152.6 to 217 (p=NS), penicillins+inhibitors from 133.9 to 210.9 ($p = 0.028$), carbapenems from 3.8 to 18 ($p = 0.005$), aminoglycosides from 45.4 to 35.2 (p=NS), macrolides from 35.9 to 68.2 (p=NS), quinolones from 28.6 to 109.6 ($p < 0.001$) and glycopeptides from 1.3 to 22.7 DDDs per 1000 patient days ($p = 0.002$). It is worth noting that carbapenems, quinolones and glycopeptides are included in the restricted antibiotics list for all Hellenic hospitals since late ’80s, together with 3rd-4th generation cephalosporins/monobactams.



Conclusions: During the study period, total consumption increased significantly and this was the case for almost all major classes of antibiotics as well, including ones under restriction. Future studies will reveal if the decrease of total consumption recorded in 2006 compared to 2005 is incidental or is the beginning of a downwards move of the consumption. Meanwhile, due to the incremental trend of total and restricted antibiotics consumption, it is quite obvious that our restriction policy is unsuccessful and should be revised.

P1272 Demographic factors associated with increase in antibiotic consumption in Danish primary healthcare

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Objectives: From 1997 to 2006 an increase of 24% in total antibiotic consumption in Danish pri-mary healthcare has been observed. The aim was to compare and possibly associate various demo-graphic factors with the increase in antibiotic consumption.
Methods: Data were obtained from the DANMAP 2006 report, the Danish Medicines Agency, the National Board of Health and the Statistics Denmark.
Results: The antibiotic consumption varied between the 15 Danish counties; however the increase was parallel in all counties throughout the study period. From 2000–2006 the increase in number treated/1000 inhab./days was 6% for both women and men. A significantly higher proportion of women received antibiotics (358/1000 inhab.) compared to the proportion of men (261/1000 in-hab.). People of both gender aged >70 years received 1.6 times more antibiotics than people <70 years of age.
The distribution by sex in the population did not change throughout the study period.
From 1997 to 2006 the population has increased by 145,000 inhabitants to a total of 5.4 million. The major part of this increase was in the age group; 60–69 years of age (~125,000). An estimated extra antibiotic consumption of these additional 125,000 people would only increase the total use of antibiotics in 2006 by 4%. From 1997 to 2006 the overall number of contacts with primary healthcare – which can be used as a general measure of the activity in primary healthcare – increased 38% for women and 32% for men.
During the period the major relative increase in consumption of individual antibiotic groups was in β-lactamase resistant penicillins (209%) and β-lactam-inhibitor combinations (500%), but these groups only constituted 6.9 and 0.8% of the total use, respectively. The consumption of the largest group, β-lactamase sensitive penicillins representing 35% of the total consumption in primary healthcare throughout the entire study period, increased 18%.
Conclusion: The 24% increase in total antibiotic use from 1997 to 2006 in Denmark was parallel in all counties and parallel for women and men at all age groups. While the total population increased only slightly, a 32–38% increase in contacts with primary healthcare was observed during the pe-riod. The overall increase encompassed most antimicrobial groups. Apparently, the recent increase in antibiotic consumption in Denmark is due to general socio-health factors with no easy single tar-gets for intervention.

P1273 The intravenous route in hospital patients – Is this being misused?

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Aims: It is assumed that almost every patient who is admitted to hospital will have a peripheral cannula inserted. It is observed that patients receive intravenous therapy long after resuming a normal gut function. The aim of this study was to assess if intravenous therapy could be terminated sooner thereby avoiding the associated costs and increased patient morbidity
Methods: 200 randomly selected medical and surgical hospital in-patients across three NHS Trusts in Southern England who had a peripheral venous cannulae inserted for intravenous therapy were monitored daily for 8 days or until removal of the cannula – whichever came first. A non-interventional study was undertaken by gathering information on all aspects of intravenous therapy including intravenous fluids and antibiotics that the patient received. The ability of the patient to take a normal diet was also checked – this signifying that the patient had a normally functioning gut, which could absorb oral fluids and medicines, whereby, use of the intravenous route could have been avoided.
Results: 74% of cannulae continued to be used in patients to give intravenous fluids and antibiotics inspite of the patient being on a normal diet. 40% of the cannulae were left in for more than 48 hours after their use had stopped with a minority being left in for more than 120 hours. The tendency for healthcare personnel to continue patients on intravenous fluids and medication in spite of the patient on a normal diet was largely attributed to the lack of awareness among the healthcare personnel
Discussion: This study highlights an urgent need to increase the awareness among the healthcare personnel. The ability of a patient to fully absorb oral medications while on a normal diet is being stressed along with the need to increase oral fluid intake of the patient instead of continuing on intravenous fluids. Though peripheral cannulae are essential in patients, their use should be regulated and carefully monitored.
Peripheral intravenous cannulation is an innocuous procedure but has a potential for significant morbidity and increased healthcare costs. The use beyond necessity remains singularly unrecognised. Attention to detail and continuing education of the healthcare personnel is all important.

P1274 Factors affecting achievement of antimicrobial prescribing targets in general practice

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Objectives: During the last decade, the emergence and spread of antimicrobial resistance has become a major global healthcare concern. There is a plethora of evidence showing that antimicrobial consumption is the major factor driving resistance and a number of intervention programmes have been initiated internationally. Of central importance to these programmes are measures to reduce inappropriate antimicrobial use. Following government recommendations to facilitate reduced prescribing, in 2001, the health authorities in Wales introduced antimicrobial prescribing indicator targets as part of a general practitioner (GP) prescribing incentive scheme. The aim of this study is

to investigate factors affecting achievement of antimicrobial prescribing targets by GPs in Wales.

Methods: 480 general practices comprising 1785 GPs based in Wales were included in this cross-sectional study. Antimicrobial prescribing analysis and cost (PACT) data for 2001 was scored against the prescribing indicator targets for the year (see table). A score of zero was interpreted as 'failing to achieve' the target. Logistic regression modelling was used to explore the relationship between outcome and possible explanatory variables including age or gender of GP, contracted hours, place of qualification, number of GPs in practice, list size (patients per GP), deprivation index (Townsend score), dispensing practice, morbidity data, and practice training status.

Results: Overall, the percentages of practices failing to meet Target 1 (winter), Target 1 (summer), Target 2, and Target 3 were 14.6, 8.5, 11.9, and 45.8 respectively. Practices that failed to achieve Target 1 (winter) were situated in areas of high-deprivation where practitioners were single-handed, not-qualified in the UK and near retirement ($p < 0.001$). However, for the summer quarters failure was associated with training practices in areas of low-deprivation ($p < 0.05$). Failure in both Targets 2 & 3 was most strongly associated with dispensing practices ($p < 0.05$).

Conclusions: The factors associated with failing to achieve the targets are complex with different practice profiles for those failing summer and winter targets. This may relate to the nature of the targets which suggests that great care should be taken in target design. The financial incentives associated with being a dispensing practice appear to skew the prescribing practices of GPs to increased use of fluoroquinolones and less use of narrow spectrum agents.

P1275 Trends in antibiotic prescription among children attending daycare centres in France: 1999–2006

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Objectives: to assess trends in antibiotic prescription among children attending day-care centres (DCC) following promotion of prudent antibiotic use (PAU) in uncomplicated paediatric respiratory tract infections (RTI).

Methods: antibiotic prescriptions were recorded from health booklets over a 3-month period preceding each of 4 pneumococcal nasopharyngeal carriage surveys among a random sample of children from January-March 1999, 2002, 2004, 2006 in DCCs in the Nord (ND) and Alpes Maritimes (AM) areas. A local intervention promoting PAU in paediatric RTI was conducted in AM in 2000 and 2003, via academic detailing visits to all general practitioners and paediatricians, and a national media campaign began in 2002. Number of treated episodes and distribution of diagnoses were studied for each survey in both areas.

Table 1: Trends in proportion of treated children

Year	N	% treated	OR	95% CI	p
Alpes Maritimes					
1999	221	59.30%	1		
2002	209	48.80%	0.65	0.44, 0.98	0.03
2004	258	48.80%	0.66	0.45, 0.96	0.02
2006	283	25.40%	0.23	0.16, 0.35	$<10^{-3}$
Nord					
1999	226	74.80%	1		
2002	177	65.50%	0.64	0.41, 1.01	0.04
2004	171	55.00%	0.41	0.26, 0.64	$<10^{-3}$
2006	227	50.20%	0.34	0.022, 0.52	$<10^{-3}$

Results: The proportion of treated children fell significantly over the study period (Table 1), as did the average number of treated infections/child, from 1.00±0.07 to 0.33±0.65 in 2006 in AM and from 1.57±1.26 to 0.84±1.09 in ND between 1999 and 2006, respectively ($p < 10^{-5}$ for each area). Among documented diagnoses, the proportion of prescriptions for rhinopharyngitis decreased from 15.1% to 6.5% in AM ($p = 0.08$) and from 24.4% to 13.7% in ND ($p = 0.06$), while that for acute otitis media increased from 34.2% (N=193) to 45.7% (N=81) in AM ($p = 0.07$) and from 31.6% (N=291) to 53.6% (N=168) in ND ($p < 10^{-5}$) between 1999 and 2006, respectively.

Distribution of antibiotic classes between 1999 and 2006 shifted towards 3rd generation cephalosporins from 25.8% of prescriptions (N=217) to. 37.5%(N=104) in AM ($p = 0.03$) and from 19.0%(N=358) to. 40.8%(N=191) in ND($p < 10^{-5}$), respectively, at the expense of 1st generation cephalosporins and macrolides.

Conclusion: Interventions advising PAU proved effective in RTI management among children attending DCCs. Fewer children were treated and rhinopharyngitis accounted for a lower proportion of prescriptions after the interventions. However, increasing use of wide spectrum antibiotics may hamper efforts to curb bacterial resistance.

P1276 Role of different classes of antimicrobials on the risk of acquisition of resistant micro-organisms in critically ill medical patients

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Objective: To assess the role of different classes of antibiotics on the risk of acquisition of resistant micro-organisms in critically ill medical patients.

Methods: From March 2006 to May 2007, all patients admitted to an 8-bed medical intensive care unit (MICU) were subjected to swabbing of nares, pharynx and rectum, and culture of respiratory secretions on admission and thrice weekly thereafter. Acquisition of a resistant microorganism was defined as the isolation, after 48 hours of MICU stay, of a strain of meticillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci, enteric Gram-negative bacilli resistant to third-generation cephalosporins, *Pseudomonas aeruginosa* resistant to at least one class of antipseudomonals or any other non-fermenter from patients whose admission cultures were either negative or positive for a susceptible isolate of the same species. The first acquired resistant microorganism defined the outcome. Recorded clinical variables included demographics, severity scores, underlying conditions and exposure to any procedure or drugs during MICU stay. Analysis was restricted to patients with a length of stay greater than 2 days and performed by using a logistic regression procedure.

Results: Out of 293 patients evaluated, 72 (25%) acquired a resistant microorganism, including a Gram-positive in 8 (3%), *P. aeruginosa* in 19 (6%), other non-fermenters in 12 (4%), *Escherichia coli* in 16 (5%), *Klebsiella* spp. in 6 (2%), and an enteric ampC-bearing organism in 11 (4%). The primary site of colonisation involved the rectum in 57 cases (79%). Of 270 (92%) patients exposed to antimicrobials, 204 (76%) received an antipseudomonal regimen, including ceftazidime in 33 (12%), piperacillin-tazobactam in 62 (23%), meropenem in 93 (34%) and a quinolone in 106 (39%, ciprofloxacin in 33 and levofloxacin in 73). Multivariate analysis selected age (OR 1.02, 95% CI 1–1.04), use of ceftazidime for >3 d (OR 3.8, 95% CI 1.3–11), mechanical ventilation for >3 d (OR 7.9, 95% CI 2–31) and exposure to a nasogastric tube (OR 15, 95% CI 2.8–79) as the best predictors for the acquisition of resistant organisms, whereas location in two particular rooms was protective (OR 0.3, 95% CI 0.1–0.8).

Conclusions: Currently, in critical care settings characterised by an almost universal exposure to antimicrobials, ceftazidime use may represent the antimicrobial exposure associated with the highest risk for the acquisition of resistant bacteria.

P1277 Pharmaceutical quality and supply route of antibiotics obtained with or without prescription (over the counter) in Indonesia

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Background: Our survey of 2996 individuals visiting public healthcare facilities in Indonesia showed that 17% of antibiotic users obtained the antibiotics over the counter (OTC) from different suppliers. Use of amoxicillin, ampicillin, chloramphenicol, ciprofloxacin, cotrimoxazole and tetracycline was most frequently reported.

Objective: To determine the supply route and the pharmaceutical quality (PQ) of frequently used oral antibiotics outside hospitals.

Methods: Four simulated clients requested antibiotics in 3 areas surrounding a government hospital and 2 healthcare centres in Surabaya. Clients went in 4 directions to visit each supplier on their journey in order to purchase 20 samples of 5 antibiotics. Sampling was equally divided over the different areas and suppliers. Samples were photographed, including package. PQ of tablets and capsules was analysed by a certified independent Dutch laboratory using HPLC.

Results: 75 pharmacies, 10 drugstores and 40 roadside stalls (kiosks) were visited. 104 samples were obtained, 76% without prescription and 81% generic. All but one out of 14 manufacturers were Indonesian. Never was oral information provided. Written information was limited to a copy of the dosing regimen from the prescription. Although “by doctor’s prescription only” was printed on all blisters, none of the OTC requests were refused nor questioned. Only one drugstore sold antibiotics (tetracycline); 29 kiosks sold either amoxicillin, chloramphenicol, ciprofloxacin or tetracycline. The PQ of 23 chloramphenicol and 19 ciprofloxacin samples were within British Pharmacopeia (BP) limits. The content of 5/20 amoxicillin and 5/22 tetracycline samples were <5% below BP limits, but in 10/20 of cotrimoxazole tablets, trimethoprim was 20% below BP limits, 8/9 of which from the same manufacturer. There was no difference of PQ between suppliers, between generic and branded samples, or between blister-packed and unpackaged (plastic bag) samples. Prices of antibiotics from the same manufacturers were invariably higher in kiosks than in pharmacies; prices per tablet/capsule varied up to 6 fold for cotrimoxazole to 20 fold for ciprofloxacin.

Conclusion: Substandard quality is probably a manufacturing problem. Although in Indonesia OTC sales of antibiotics are prohibited by law, antibiotics were sold OTC in pharmacies and some were retailed at higher prices in kiosks. Enforcement of legislation is urgently needed.

P1278 The effect of antimicrobial use on resistance in *Escherichia coli* in Finland

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Objectives: The connection between regional antimicrobial resistance rates in *Escherichia coli* and regional outpatient antimicrobial use in Finland was investigated.

Methods: During a 9-year study period of 1997–2005, more than 700 000 *E. coli* isolates, mainly from urine samples, were tested for antimicrobial resistance in 26 microbiological laboratories, representing 18 of the 20 central hospital districts in Finland. The following antimicrobials were included in the study: amoxicillin, amoxicillin-clavulanate, first-generation cephalosporins (mainly cephalexin), fluoroquinolones (of which levofloxacin, norfloxacin, and ciprofloxacin were studied separately), trimethoprim, sulfatrimethoprim, pivmecillinam, and nitrofurantoin (Table). There were two study approaches: (1) the level of antimicrobial use of one year was compared with the level of resistance of the next year, and (2) the change in antimicrobial use was compared with the change in resistance within the study period.

Results: The following significant associations were found between the level of use and the level of resistance (approach No. 1): nitrofurantoin use vs. nitrofurantoin resistance, cephalosporin use vs. nitrofurantoin resistance, amoxicillin use vs. fluoroquinolone resistance, fluoroquinolone use vs. ampicillin resistance. The following significant associations were found between the change in use and change in resistance (approach No. 2): amoxicillin or amoxicillin-clavulanate use vs. ampicillin resistance, combined use of trimethoprim and sulfatrimethoprim vs. trimethoprim and sulfatrimethoprim resistance, and trimethoprim use vs. sulfatrimethoprim resistance. No other positive connections were found (Table).

Level of resistance and consumption			Change in resistance and consumption		
Resistance		Consumption	Resistance		Consumption
Ampicillin	vs.	Amoxicillin	Ampicillin	vs.	**Amoxicillin**
		Amoxicillin + Clavulanate			**Amoxicillin + Clavulanate**
		Fluoroquinolones			Fluoroquinolones
Cephalothin	vs.	Cephalosporins	Cephalothin	vs.	Cephalosporins
		Fluoroquinolones			Fluoroquinolones
		Trimethoprim			Trimethoprim
		Sulfatimethoprim			Sulfatimethoprim
		Trimethoprim + Sulfatrimethoprim			Trimethoprim + Sulfatrimethoprim
		Nitrofurantoin			Nitrofurantoin
Fluoroquinolones	vs.	**Amoxicillin**	Fluoroquinolones	vs.	Amoxicillin
		Amoxicillin + Clavulanate			Amoxicillin + Clavulanate
		Cephalosporins			Cephalosporins
		All fluoroquinolones			All fluoroquinolones
		Levofloxacin			Levofloxacin
		Norfloxacin			Norfloxacin
		Ciprofloxacin			Ciprofloxacin
Trimethoprim	vs.	Trimethoprim	Trimethoprim	vs.	Trimethoprim
		Sulfatrimethoprim			Sulfatrimethoprim
		Trimethoprim + Sulfatrimethoprim			**Trimethoprim + Sulfatrimethoprim**
Sulfatrimethoprim	vs.	Trimethoprim	Sulfatrimethoprim	vs.	**Trimethoprim**
		Sulfatrimethoprim			Sulfatrimethoprim
		Trimethoprim + Sulfatrimethoprim			**Trimethoprim + Sulfatrimethoprim**
Mecillinam	vs.	Pivmecillinam	Mecillinam	vs.	Pivmecillinam
Nitrofurantoin	vs.	**Cephalosporins**	Nitrofurantoin	vs.	Cephalosporins
		Nitrofurantoin			Nitrofurantoin

Conclusion: Because of the link between nitrofurantoin consumption and resistance, more attention should be paid to the prescription of nitrofurantoin for outpatient *E. coli* infections. An interesting cross-connection seems to exist between amoxicillin use vs. fluoroquinolone resistance and fluoroquinolone use vs. ampicillin resistance. Interestingly, a connection was not found between fluoroquinolone use and fluoroquinolone resistance.

P1279 Relationship between antimicrobial use and antimicrobial resistance in *E. coli* and *Klebsiella pneumoniae* blood isolates at 5 tertiary hospitals in Korea

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Objectives: Increases in the prevalence of antibiotic-resistant Gram-negative bacilli (GNB) in hospitals are frequently related to the high selective pressure of antibiotics. This study was performed to evaluate the association between antimicrobial resistance in *E. coli* & *Klebsiella pneumoniae* that caused bacteraemia in hospitalised patients and anti-GNB antimicrobial use in 5 tertiary teaching hospitals in Korea.

Methods: Data on annual patient-days and consumption (defined daily dose (DDD) per 100 patient-days) of antibiotics from January 2005 to December 2005 in 5 tertiary hospitals in various regions of Korea were analysed. A total 302 nonduplicate *E. coli* & *K. pneumoniae* isolates that caused nosocomial bacteraemia was collected from July 2006 to November 2006. To determine the antimicrobial resistance, broth microdilution test and double disk synergy were performed according to the CLSI performance standards. Spearman’s correlation coeffient was used to determine the relationship between antibiotic consumption and resistance.

Results: The prescription of anti-GNB antibiotics in 5 tertiary hospitals markedly varied (median value 93.04 DDD/ 100 patients-days, range 34.81–117.78 DDD/ 100 patients-days). Ceftazidime-resistant *E. coli*

& *K. pneumoniae* was significantly correlated with the higher use of aminopenicillins (r=0.900, $P<0.05$), total aminoglycosides (r=0.900, $P<0.05$), and carbapenems (r=0.900, $P<0.05$). Cefotaxime resistance showed positive correlation with the higher use of aminopenicillins and ceftazidime, but had no statistical significance (r=0.718, $P>0.05$ and r=0.600, $P>0.05$, respectively). Imipenem resistance showed week positive correlation with the higher use of carbapenems, but had no statistical significance (r=0.671, $P>0.05$).
Conclusion: The higher consumption of some antibiotics was significantly correlated with the higher rate of antibiotic-resistant *E. coli* & *K. pneumoniae*. Continuous surveillance of antibiotic use is needed to encourage appropriate prescribing of antibiotics and to reduce antibiotic resistance.

P1280 Junior doctors' perceptions of the problem of bacterial resistance in hospitals

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Objectives: The aim of this study was to assess how the problem of bacterial resistance was perceived by the junior doctors.
Methods: We surveyed 63 junior doctors in their first year of postgraduate training at one university teaching hospital in Dundee (Scotland, UK) to assess their attitudes about the importance of antibiotic resistance, knowledge of its prevalence, beliefs about its causes and attitudes about interventions designed to address the problem.
Results: The response rate was 70% (63 of 90 doctors). Thirty-five (56%) junior doctors were training in a medical speciality and 24 (38%) in a surgical one. Sixty-two (98%) of them had prescribed an antibiotic within the past 6 months. Antibiotic resistance was perceived as a national problem by 92% of the respondents, but only 52% rated the problem as important in their own daily practice. Only 22% of the junior doctors had a correct idea of the prevalence of MRSA in UK hospitals. Nearly all doctors (94%) believed that widespread and inappropriate antibiotic use, or prescription of broad spectrum antibiotics were important causes of resistance. Excessive durations of antibiotic treatments were rated as an important cause of resistance by 80% of the doctors, but only 51% recognised too low antibiotic doses as a potential cause of bacterial resistance. Finally, only 45% of the doctors identified poor hand hygiene practices as an important driver for resistance. More than 90% of the junior doctors rated the following measures as helpful to improve antibiotic prescribing: educational sessions, availability of guidelines and readily accessible advice from a microbiologist/an Infectious Diseases specialist/a pharmacist/or a colleague. Regular audit and feedback of the antibiotic prescribing practices on the ward was found helpful by 82% of the doctors and availability of resistance data was rated useful by 77% of them. Restrictive measures were not as popular, with 66% of the junior doctors rating restriction of some antibiotics useful, and 13% finding the restriction of all antibiotics potentially helpful.
Conclusion: Although most junior doctors view antibiotic resistance as a serious national problem, perceptions about its local importance, its causes, and possible solutions vary more widely and are not always evidence-based. These perceptions must be taken into account to maximise adherence of the junior doctors to the measures aiming at curbing bacterial resistance.

P1281 Outpatient utilisation of antibiotics in Zagreb, 2006

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Objectives: Antibiotics accounted for 4% of total prescription drug utilisation in 2006, expressed as DDD/TID, and for 10% of financial drug utilisation. The aim of the study was to assess antibiotic utilisation in 2006 in the City of Zagreb.
Methods: Data on the number of packages for each individual drug were collected from all Zagreb Pharmacy units. Based on these data, the number of defined daily doses(DDD)and DDD/1000inhabitants/day(DDD/TID)for 2006 were calculated for all 14 drug groups of the Anatomical-Therapeutic-Chemical (ATC) drug classification system at all levels of ATC system for each individual drug.
Results: Total outpatient utilisation of antibiotics in Zagreb was 37.38DDD/TID, with penicillins accounting for 50%.The subgroup of penicillin combinations yielded a 54% share in the utilisation of antibiotics, and referred completely to amoxicillin + clavulanic acid combination, for years the most frequently prescribed antibiotic in Zagreb. The subgroup of penicillins sensitive to β-lactamases, accounted for 23% of penicillin utilisation. The subgroup of broad-spectrum penicillins, accounted for 21.50% of penicillin utilisation. The majority of this subgroup utilisation referred to amoxicillin with 3.96 DDD/TID. The subgroup of β-lactamase resistant penicillins showed lowest share in the overall antibiotic utilisation. Cephalosporins accounted for 20.5% of total antibiotic utilisation. Cefuroximeaxetil accounted for the most part of utilisation with 5.5DDD/TID, followed by cephalexin with 1.44DDD/TID. In the J01E subgroup, sulfamethoxazole+trimethoprim accounted for the entire utilisation with1.39DDD/TID.The utilisation of macrolides was 4.04 DDD/TID.Aminoglycosides showed a utilisation of 0.15DDD/TID.Tetracyclines accounted for 7%, and quinolones, for 6% of antibiotic utilisation in Zagreb. Norfloxacin accounted for 70% of utilisation in this group. In the group of other antimicrobials, nitrofurantoin accounted for almost entire outpatient utilisation with 0.90 DDD/TID.
Conclusion: Almost one-third of outpatient antibiotic utilisation in the City of Zagreb in 2006 referred to amoxicillin+clavulanic acid, whereas current guidelines suggest that narrow-spectrum antibiotics should be preferred. Therefore, the utilisation of antibiotics in the City of Zagreb could not be considered appropriate.

Antibiotic usage and abusage: the solutions

P1282 Results after the introduction of a pharmacist-managed surgical antibiotic use policy

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Objectives: Our study aims at comparing the use of surgical antibiotics before and after the introduction of local surgical antibiotic use policy.
Methods: The research was performed at an 82-bed General Surgery Department. The local surgical antibiotic use policy was launched by the pharmacy service in December 2005. We analysed the surgery-related antibiotic use in the February months the preceding (2004–2005: Period I) and in the subsequent two years (2006–2007: Period II). In order to determine individual antibiotic usage, data were collected retrospectively from flow-charts and anaesthetic records. Surgical procedures were classified according to wound class categories. The following parameters were compared: the frequency of antibiotic use in different wound class categories, the timing of antibiotic administration, the duration and the average price of antibiotic use.
Results: 822 operations were performed (438 in period I; 384 in period II). In terms of wound class, antibiotics were administered in the following proportions: 30.3% vs. 44.8% clean, 54.2% vs. 50.0% clean-contaminated, 41.3% vs. 60.3% contaminated, and 86.2% vs. 83.3% infected surgeries. The most frequently used antibiotics was cefuroxime (or cefuroxime-metronidazole combination) in both periods. Concerning proper timing, Period I saw antibiotics administered in the morning, disregarding whether the patient was operated immediately afterwards or only later during the day. In period II antibiotics were administered in the operating theatre in all instances, concurrently with the induction of anaesthesia. The average duration of the surgery-related antibiotic use was 3.3±2.0 days in period I, vs. 2.4±2.0 days in period II. Calculating with prices valid in 2007, the average price of surgery-related antibiotic use has decreased from 18.3±5 to 9.0±2 Euro.
Conclusion: The introduction of the local surgery-related antibiotic use policy was successful in several aspects, since proper timing, shorter duration of prophylactic antibiotic use and cost savings were achieved. However, patient-level analysis highlighted fields of inappropriateness:

despite the fact that prophylactic or therapeutic antibiotic use would be in several cases the surgeons did not indicate antibiotic use. Therefore, we demonstrated that the initiations of the pharmacy service and the continuous evaluation of its effectiveness are both essential in order to improve surgery-related antibiotic use.

P1283 Implementation of an intravenous to oral switch policy for antimicrobials in a secondary care setting

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Objectives: Sequential therapy is defined as the timely transition from parenteral to oral antimicrobial therapy. It is promoted as intravenous therapy is more expensive, has significant adverse effects, is time consuming for staff and is not always more efficacious.
To develop and issue guidelines to clinical pharmacists on enacting an intravenous to oral switch policy in a large Irish teaching hospital.
To implement an intravenous to oral switch policy, and to examine the effect of this policy.
To find out if an earlier switch to an oral antimicrobial resulted in a shorter hospital stay.
To record where empiric treatment was prescribed, if the initial antimicrobial chosen complied with the hospital empiric guidelines.
To calculate cost savings generated from the implementation of the stategy.
Methodology: This was a prospective, controlled before and after study. A control and study group were included. The intervention consisted of the application to the drug chart of stickers promoting an oral switch along with a guide on the criteria for switch. Duration of intravenous treatment, the number of days after the criteria were met before antimicrobials were switched to oral, length of stay and cost savings were measured. Compliance with the hospital empiric antimicrobial guidelines was assessed.
Results: The study included 236 patients prescribed 289 intravenous antimicrobial courses. The duration of intravenous treatment reduced significantly between the pre- and post-intervention periods, when comparing the study group to the control group ($p = 0.02$). The number of days after the criteria for switching was met before the patient was switched to oral antimicrobials reduced significantly post-intervention in the study group compared to the control group ($p = 0.017$). The median length of stay did not reduce. The intervention was cost effective, and resulted in some cost savings, (€6.41 per patient in the post-intervention study group). 88.8% of prescriptions were concordant with the empiric antimicrobial prescribing guidelines.
Conclusions: Implementation of the strategy resulted in a reduced duration of intravenous antimicrobial treatment, with resulting cost savings. There was no reduction in the median length of stay, likely due to social reasons and other co-morbidities lengthening stay. This strategy is to be rolled out to all general medical and surgical wards in the hospital.

P1284 The Going Home project: implementing a frontline pharmacist-led team approach towards appropriate IV to oral antibiotic switch for patients with antibiotic resistant infections and subsequent discharge to outpatient management

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Objectives: A previous study showed that 29% of patient on IV glycopeptides, predominantly used to treat proven or suspected MRSA, fulfilled clinical criteria for switch to oral antibiotics and discharge to completer treatment as an outpatient with a median reduction in stay of 5 days. Prior to this study there was no IV-oral switch program for glycopeptide use with patients usually remaining in hospital until the end of treatment. The objectives of this work were to assess whether the predicted benefit of earlier appropriate discharge to home treatment could be implemented by targeting this group of patients, and if so, whether this was an effective management strategy.
Methods used: We implemented a study using the same approach. All patients on IV glycopeptides were screened against pre-determined IV-oral switch and discharge on oral antibiotics criteria. The study team identified patients suitable for IV-oral switch and discharge. Advice was provided to prescribers on the most appropriate oral antibiotic for switch and treatment at home. Follow up was arranged as appropriate to clinical need.
Switch from IV glycopeptides to oral suitable antibiotics, reduction in length of stay, IV line use, and estimated cost savings were measured. In addition pharmacist time and interventions profile was assessed.
Results: 81 (52%) of patients on IV glycopeptides were switched to an oral antibiotic. 64 (41%) were discharged home early on oral antibiotics. 22 different combinations of oral antibiotic that were either consistent with trust policy or specifically approved by senior microbiology/infectious diseases specialists were used. Overall this intervention resulted in a reduction of 1215 bed days and a further 511 in-patient IV line days. The study ran in parallel and was complimentary to the introduction of an OPAT service.
Conclusion: A targeted approach by a dedicated team results in a significant reduction in hospital length of stay for patients being treated for resistant Gram-positive infections with IV glycopeptides. This intervention improves the quality of infection management and resource utilisation. This has resulted in a successful business case for an enhanced antibiotic pharmacist team.

P1285 Unexpected impact of infectious diseases division interventions on antibiotic use in two intensive care units

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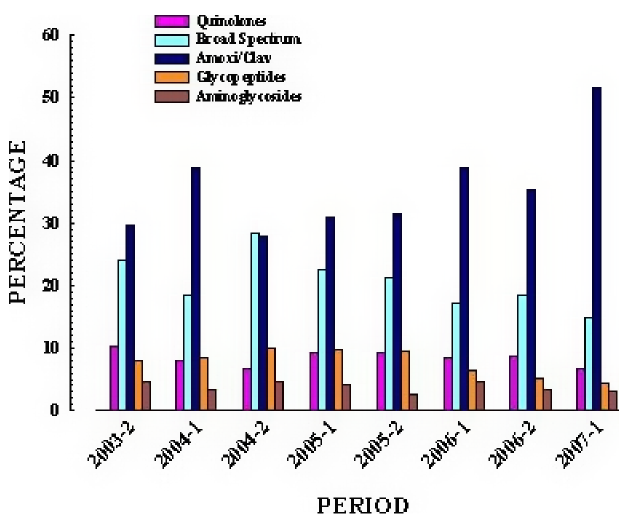
Objectives: The purpose of the present study was to estimate the impact of infectious diseases division interventions on the antibiotic use in two intensive care units (ICU) (12 beds of medical intensive care (MIC) and 12 beds of surgical intensive care (SIC) in a Belgium University hospital between 2003 and 2007.
Methods: One infectious diseases specialist with one microbiologist specialist constituted the infectious disease division and their main goals were twofold: 1) a reduction in the amount of broad spectrum antibiotics (including 3rd generation cephalosporins, cefepim, meropenem, aztreonam and piperacillin/tazobactam), fluoroquinolones, glycopeptides and aminoglycosides administered; 2) a cutback on the length of empirical treatment. The interventions started in 2005 with quality rounds (twice a week).
Antibiotic consumption data were obtained from the pharmacy and expressed as the number of DDDs (Daily Dose Defined) per 100 bed days.



Results: The interventions of the infectious diseases division resulted in an increase of total antibiotic consumption in SIC from 224 DDDs/100

bed days in 2005 to 281 in 2007 whereas a decrease from 492 to 334 DDDs was observed in MIC during the same period. In both intensive care units, the use of amoxicillin/clavulanic acid increased, particularly in SIC from 65 to 139 DDDs, while the proportion of broad spectrum antibiotics given decreased significantly. Aminoglycosides remained stable in both ICU whereas glycopeptides and fluroquinolones decreased in MIC.

Conclusions: The overall antibiotic consumption in SIC increased dramatically due to a progressive evolution towards a polyvalent intensive care with a mixture of surgical and medical pathologies. The pressure exerted by the intervention division on the ICU physicians led to a decrease use in broad spectrum antibiotics but at the expense of a spectacular increase in the consumption of amoxicillin/clavulanic acid. These preliminary data encouraged us to continue the collaboration with intensive care physicians and should prompt us to think on the real need of antibiotic therapy even in the ICU setting.

P1286 Checklist for an optimal early switch to oral antibiotics in hospitalised patients

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Background: Several studies have evaluated early switch criteria from intravenous (IV) to oral antibiotic therapy in restricted patient populations, e.g. with community-acquired pneumonia. Only few reliable data are available about using switch criteria in general medicine wards.

Objectives: To evaluate the effect of a checklist for early switch from IV to oral antibiotics in general medicine wards on duration of IV antibiotic therapy, safety and costs.

Methods: The study was performed in general medicine wards with 2500 admissions annually in a university hospital. All patients on IV antibiotics were enrolled. A checklist with 10 switch criteria such as defined diagnoses, clinical improvement with defined defervescence and oral suitability of antibiotics was placed in the charts. We analysed the checklists of the entire study-year (8/06 to 7/07) providing information about reasons against oral switch and safety aspects (n = 398). In a pre-post analysis we compared the outcome parameters in the inventorial phase (April-July 2006, n = 376) to the implementation phase (April-July 2007, n = 350).

Results: A significant reduction of 1d IV therapy was achieved (6 to 5d in median, p = 0.005) without any increase of relapses, readmissions or death. The crude relative reduction of days of IV antibiotic treatment was 16% (95% CI 4–26%, p = 0.01) and 19% (9%-29%, p = 0.001) after adjustment for age, gender, Charlson index, presence of malignancy and consultation by infectious disease specialist. The main reasons not to switch on day 3 of IV therapy were persisting fever (41% of non-switched patients) and absence of clinical improvement (41%). Two of 698 patients experienced a relapse after early switch; both responded to continued IV antibiotic treatment. A total of 20,000€ of acquisition costs for antibiotics were saved in the year of the intervention in contrast to a 12.5% (+30,000 €) increase of acquisition costs the year before (2005 to 2006). The workload for IV applications was reduced by about 350 hours. There was no similar decrease in antibiotic cost on control wards during the study period.

Conclusions: The implementation of a checklist with clearly defined switch criteria leads to a significant reduction of IV therapy and costs in a university hospital. The proposed bedside checklist can be used safely regarding relapses, readmissions and fatality rate in a mixed population on medical wards.

P1287 Antimicrobial stewardship programme may help reduce antimicrobial resistance in the intensive care unit

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Objectives: Resistance (R) to antimicrobials (AB) is one of the most challenging issues in the management of ICU patients (pts); inappropriate use of AB plays a major role in increasing R.

Methods: from Jan 2002 to Dec 2006, a prospective survey of R patterns among the most important nosocomial pathogens was performed in pts admitted in ICU >48–72 hrs. From 2003, an antimicrobial stewardship program (ASP) has been implemented with the following interventions: I) elimination of 3rd-generation cephalosporin prophylaxis in critical pts at the time of admission to ICU; II) administration of empirical AB therapy of suspected ICU-acquired infections based both on local epidemiology of R and pharmacokinetic/pharmacodynamic (PK/PD) parameters; III) regular (three times a week, or on-call basis) re-evaluation of AB therapy, aiming at stepping-down and shortening duration.

Results: from 2002 (as historical reference) to 2006, the following important decreases in antimicrobial consumption, evaluated in defined daily doses (DDD), have been recorded through the ESGAP ABC Calc 3.1b program: AMK –93.1%; SAM –57.7%; CIP –45%; GEN –88.4%; IPM –60.5%; TEC –95%; VAN –73.3%; CAZ –78.6%. In the same period, MEM and LZD consumption strongly increased (+1620%, and +487%, respectively). Contemporarily, an increase in susceptibility of *S. aureus* to OXA and CIP (from 65.5 to 84.2%, and from 65.7 to 84.3%, respectively), and of *P. aeruginosa* to TZP (from 88.8 to 93.5%), CAZ (from 82.7 to 90.3%), FEP (from 87.5 to 93.5%), AMK (from 93 to 100%) have been observed. However, susceptibility of *P. aeruginosa* to IPM decreased from 75.3 to 45.2%, and production of ESBL increased in *E. coli* and *K. pneumoniae* (from 3.6 to 11.3 and 5.6%, respectively). AB costs did not increase from 2002 to 2006 (109,627.07 and 115,492.63 Euros, respectively) despite a similar number of pts undergoing AB therapy and increased consumption of very expensive AB, such as LZD (49,268 Euros only in 2006).

Conclusion: The ASP yielded to progressive reduction in R patterns of *S. aureus*, and of *P. aeruginosa* to the most important AB. Despite an increased consumption of newer AB, due to an innovative PD-driven approach, the ASP also led to overall expenditure containment.

P1288 Influence of rapid antigen detection test use to antibiotic prescribing for tonsillopharyngitis

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Objectives: The aim of the study was to evaluate influence of diagnostic tests to antibiotic prescribing and economical benefit.

Methods: Intervention study was carried out in two randomly selected outpatient (GP) clinics. Intervention consisted of 1) modern knowledge of diagnostics and appropriate antibiotic use for treatment of upper respiratory tract infections, particularly of tonsillopharyngitis, and 2) free rapid antigen detection test (RADT) for Group A hemolytic streptococcus (GABHS), recommendations for its use and interpretation of results. All cases (463) of tonsillopahryngitis from randomly selected patients, who visited GP in 2004, were included in control group. All cases (318) of tonsillopharyngitis from the patients, who visited GP in 2006, till the needed sample size was reached were included in reference group.

Results: After implementation of RADT increase in use of other diagnostic tests was noted. General blood test was performed 1.3 times (p = 0.03), erythrocyte sedimentation rate – 5.9 times (p = 0.00) more often, C-reactive protein test (CRP; not used before intervention at all) was started. The structure of prescribed antibiotics has changed. Penicillin was prescribed 5 times, clarythromycin and cephadroxyl 2 times more often, while broad spectrum penicillins as amoxicillin and amoxicillin with clavulanic acid decreased (respectively 2 and 5 times). Antibiotic prescription rate in general hasn't changed statistically significantly, as discrepancies of prescription rates in both clinics were found – it decreased 1.4 times (p = 0.02) in one clinic, but increased 1.2 times (p = 0.27) in another clinic. Positive RADT result necessitated 23 times more frequent antibiotic prescription than its negative result. However even getting negative RADT result, GPs prescribed antibiotics to 40.4% cases. Proportions of complications and recurrences decreased (respectively 2.1 and 3.7 times). However economical benefit in treatment of tonsillopharyngitis using RADT was gained just in one clinic. Characteristics of used in study RADT were determined: sensitivity

72.3%, specificity 92.3%, positive prognostic value 86.1%, negative prognostic value 83.5%.

Conclusions: After implementation of RADT, other diagnostic tests were used more often, importance of CRP was found in differential diagnosis of tonsillopharyngitis. Prescribed antibiotic structure has changed with the substantial increase of narrow spectrum penicillin.

P1289 A controlled intervention study to improve antibiotic use in a Russian paediatric hospital

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Objective: In a paediatric hospital in Russia a controlled intervention study was performed to improve antibiotic use and to see if improvements persisted. During October, November, and December in 2002 clinical and microbiological data, antibiotic use, costs, and outcome were recorded at two wards for gastrointestinal infections (GII) and two wards for respiratory tract infections (RTI).

Methods: Guidelines of diagnosis and treatment of infections were developed and implemented at one ward for GII and one ward for RTI in 2003. The other two wards served as controls. The same data were recorded during the same 3 months periods in 2003 and 2004.

Results: At the intervention wards the percentage of patients with GII who received antibiotics decreased from 94% in 2002 to 41% in 2003, but increased to 73% in 2004. In RTI these percentages were 90% in 2002, 53% in 2003 and 83% in 2004. The proportions of patients who received antibiotics in 2004 were still lower than in 2002, risk difference (RD) 0.217, $p=0.001$ in GII, and RD 0.073, $p=0.013$ in RTI. From 2002 to 2004 there was a decrease in cephalosporin use ($p=0.021$) and an increase in penicillin use ($p=0.032$) in pneumonia. There was no difference in mortality, duration of fever or duration of hospital stay between the intervention and control wards.

Conclusion: Antibiotic use could be halved without compromising quality of patient care, but one year after the intervention the use of antibiotics approached pre-intervention levels. Strategies to sustain the effect of interventions are needed.

P1290 Impact of a positive Binax NOW *Streptococcus pneumoniae* urinary antigen test on antibiotics policy

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Objectives: To evaluate the impact of a positive Binax NOW *Streptococcus pneumoniae* urinary antigen test (UAT) on the use of a targeted anti-pneumococcal therapy for the treatment of community-acquired pneumonia.

Methods: From April 2004 to December 2005, we retrospectively analysed 96 consecutive patients admitted to the emergency room for a presumptive diagnosis of pneumonia with a positive UAT. Patients with associated meningitis were excluded. We reviewed the antimicrobial therapy used before and after the positive result of the UAT, and particularly the proportion of patients under amoxicillin.

Results: Among the 96 patients, 92 were available for the study. Mean age was 64 years (median age 61 y, range 18–99 y) and 49 patients (51%) were male. Eighty nine patients (97%) had a blood or a respiratory tract sample which, in 23 patients (25%), was positive for *S. pneumoniae*. Prior to the UAT result, 22 patients (24%) had no antibiotics, 11 (12%) received amoxicillin, 25 (27%) amoxicillin-clavulanic acid (AMC) or a third-generation cephalosporin (C3G), and 29 (32%) received an association of β-lactam with a macrolide or a fluoroquinolone. Once the physician was informed of a positive UAT, among the 22 patients not treated initially, 7 received amoxicillin secondarily, 7 had AMC or C3G, and 4 had an association. Overall, the number of patients treated with amoxicillin rose to 38 (41%), those receiving AMC or C3G rose to 30 (33%) whereas patients under an association of antibiotics decreased to 12 (13%). Among the 23 patients who had a positive sample for *S. pneumoniae*, 12 had an antimicrobial therapy with a broader spectrum than amoxicillin even once the susceptibility to β-lactams was known.

Conclusions: In case of community-acquired pneumonia at the emergency room, physicians often prefer to prescribe a first line antimicrobial therapy with a broader spectrum than amoxicillin. Although a positive UAT resulted in an increased number of patients treated by amoxicillin alone from 12 to 41 percent, it is noteworthy that in almost 60 percent of the cases antibiotic spectrum was not narrowed.

P1291 The effect of restriction of cephalosporin usage on the incidence of ESBL-producing *Klebsiella pneumoniae* in a tertiary hospital in Cape Town, South Africa

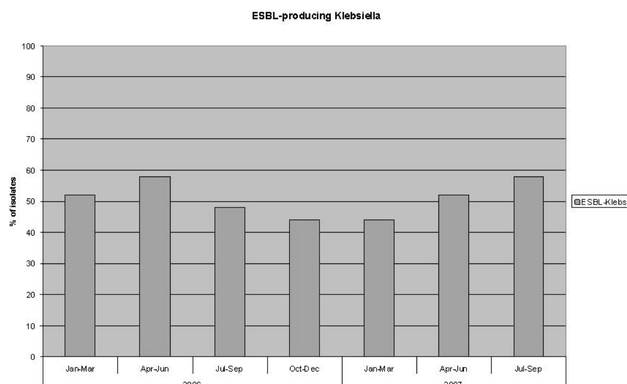
S.P. Oliver (Cape Town, ZA)

Objectives: Several studies have demonstrated a dramatic reduction in the incidence of ESBL-producing organisms when cephalosporin usage has been curtailed. This study seeks to reproduce this effect in a large teaching hospital experiencing an upsurge in ESBL-producing *Klebsiella pneumoniae*. ESBL-producing klebsiellas accounted for around 1% of laboratory isolates in 1994 and had risen to over 60% by 2005.

Methods: Antibiotic usage is controlled in this hospital by the pharmacy requiring release from a microbiologist for certain restricted agents. In May 2006 all cephalosporin usage was restricted except for the use of ceftriaxone for acute bacterial meningitis and cefazolin for surgical prophylaxis. Co-amoxiclav or piperacillin/tazobactam was made available for clinical situations where cephalosporins had previously been used.

Results: Cephalosporin usage dropped by 65%. At the same time co-amoxiclav usage increased by 468% and piperacillin/tazobactam usage increased by 252%.

ESBL-producing klebsiellas appeared to be decreasing during the second half of 2006 but have increased again during 2007. No clustered outbreak of *Klebsiella* isolates was noted at any time.



ESBL-producing *Klebsiella*

Conclusion: The decrease in the incidence of ESBL-producing *Klebsiella* in response to decreased cephalosporin usage appears not to be a sustainable phenomenon in practice.

P1292 Administration of antibiotics via the respiratory tract as monotherapy for pneumonia

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Objective: Recent studies have shown that antibiotics administered via the respiratory tract may have a role in the prevention of respiratory tract infections. However, the role of aerosolised antimicrobials administered as monotherapy for patients with pneumonia is unclear.

Methods: We endeavoured to accumulate and evaluate the published relevant evidence through searches of PubMed, Scopus, and relevant bibliographies.

Results: Seven relevant studies (1 randomised controlled trial, 4 case series and 2 case reports), including 63 patients, were identified; 37% (23/63) and 63% (40/63) of those patients suffered from community-acquired and nosocomial (including ventilator-associated) pneumonia, respectively. *Acinetobacter baumannii* (41%), Gram-positive cocci (37%), and *Pseudomonas aeruginosa* (16%) were the pathogens more frequently isolated. Colistin (49%), penicillin (37%), and aminoglycosides (17%) were the antimicrobials administered via the respiratory tract. Concurrent systemic antimicrobials (without activity against the isolated pathogens) were given to 33% (21/63) of patients. Clinical cure and bacteriological eradication were observed in 86% (54/63) and 85% (33/39) of patients, respectively. All-cause mortality was 36% (11/31).
Conclusions: The very limited published data preclude any strong conclusions. However, the available data seem to suggest that aerosolised antimicrobial monotherapy for pneumonia should not be a priori excluded in patients without intravenous access, those denying systemic treatment, and in patients with severe toxicity of systemic antimicrobial therapy. Clinicians are encouraged to publish any relevant experience in order for a considerable body of literature to be accumulated.

Bacterial pathogenesis

P1293 Virulence traits of *Escherichia coli* clinical isolates neonatal sepsis. Comparison between strains causing early and late neonatal sepsis

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Objective: Neonatal risk factors for invasive bacterial disease and its diagnosis and therapy remain an important problem for obstetricians and paediatricians. Neonatal meningitis and septicaemia caused by *E. coli* and *Streptococcus agalactiae* are still major health problems in industrialised countries. The objective of the present work was to study the pathogenicity of *E. coli* strains causing neonatal sepsis and to compare those involved in early infection and late infections.
Methods: A total of 54 *E. coli* strains causing septicaemia were collected from neonates between 0 to 28 days-old. Twenty-two and 32 strains have been caused of early or late infection, respectively. Phylogenetic group and the presence of virulence factors were determined by PCR. The type 1 fimbriae expression was tested by agglutination with a *Streptococcus cerevisiae* strain. Proportion and means were compared by using the Chi-square test and t-test.
Results: Twenty-seven (50%), 18 (33%), 6 (11%) and 3 (6%) strains belonged to phylogenetic groups B2, D, A and B1, respectively. The factors more frequently showed by the *E. coli* strains were: fimA (91%), ibe10 (61%), iucC (59%), papEF (56%) and and papC (46%); the virulence factors presented in lesser percentage were: cnf1 and sat1 (6% and 4%, respectively). The ibe10 (ibeA) gene was more prevalent in the strains involved in early infection than in those involved in late infection (86% versus 44%, respectively; p = 0.001).
Conclusion: *E. coli* strains causing early neonatal septicaemia present higher number of virulence factors than those causing late infection. The ibeA gene is more prevalent in the *E. coli* strains causing early neonatal sepsis and it could be involved in the pass of the infection to the amniotic fluid.

P1294 Phylogenetic background and virulence profile of *Escherichia coli* causing neonatal disease

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Objective: To determine the phylogenetic background and the virulence profile among *E. coli* strains causing neonatal disease, and compared with *E. coli* causing urinary bacteraemia.
Methods: Thirty-five *E. coli* strains, isolated in 4 hospitals within 1996 and 2007, 34 from blood and 1 from blood and CSF, of newborns within the first week of life. We compared them with 50 *E. coli* strains isolated from blood of patients with urinary bacteraemia (Moreno et al. 2005. Diag Microbiol Infect Dis: 53; 93). To these 85 epidemiologically unrelated strains, phylogenetic group and 15 virulence factors were determined by PCR
Results: Among the 35 *E. coli* causing neonatal disease, the highly pathogenic phylogenetic group B2 was the most frequent, 28 isolates (80%), followed by group D with 4 (11%) and group A with 3 (9%). These strains showed a high prevalence of proven virulent traits associated to extraintestinal infections, specially kpsMII (89%), malX (77%), fyuA (91%), and iutA (66%) and a relatively low prevalence of factors associated to P fimbriae: papA 43% and papG alelle II 34%. Factors associated to meningitis were ibeA 21%, and sfa/focDE 46%, but both were present in the strain isolated from CSF. In consequence, these strains showed a high aggregate virulence score with a mean of 6.8, and a range between 2 and 10 virulent factors/strain.
E. coli causing neonatal disease showed a similar distribution among phylogenetic groups than *E. coli* causing urinary bacteraemia (phylogenetic group B2 70%, group D 16%, group A 10%, and group B1 4%), a similar prevalence of virulent traits associated to extra-intestinal infections, kpsMII (76%), malX (70%), iutA (78%), and fyuA (88%), and a similar virulence score 7.2 (range, 1 to 10). However, papA and papG alelle II were statically associated to *E. coli* producing urinary bacteraemia (78% and 66%) in relation to *E. coli* producing neonatal septicaemia (P = 0.001 and P = 0.004; respectively).
Conclusions: *E. coli* producing neonatal disease derived mainly from the pathogenic phylogroup B2 and exhibited a great concentration virulent traits which inferred a virulent potential as high as *E. coli* causing urinary bacteraemia. However, P fimbriae, which enable bacteria to reach the kidney, was significant more prevalent in urinary bacteraemia.
The similarities between *E. coli* causing neonatal septicaemia, urinary bacteraemia and others, strongly suggest that all these *E. coli* should be classified as “Extraintestinal Pathogenic *E. coli* (ExPEC)”

P1295 Detection of pathogenic genes pap, hlyA and cnf1 IN *E. coli* strains isolated from adult patients with urinary tract infection

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Objective: Urinary tract infection (UTI) is one of the most commonly acquired bacterial infections in ambulatory and hospitalised populations. Most UTIs are caused by *E. coli* strains that exhibit certain virulent factors. Urovirulence factors as pap (pilus associated with pyelonephritis), hlyA (alfa haemolysin) and CNF (cytotoxic necrotising factor) play important role in the pathogenesis of *E. coli* causing of UTI. The present study was comparable in University hospital of Alexandroupolis in northern Greece. The aim was to determine the occurrence of virulence genes expressing pap, hlyA and cnf1 in adult patients with UTI.
Methods: A total 131 strains *E. coli* isolated from urines of 131 adult patients as follow: 103 subjects were women and 29 men. Fifty six strains were isolated from ambulatory and 75 from hospitalised patients. The laboratory criterion for acute *E. coli* UTI was the presence of positive culture on MC agar with of least 105 CFU/ml. The biochemical identification of the strains was performed by automated system VITEK2 (BioMerieux). Specific primers were used to amplify sequences of the pap, hlyA and cnf1 genes. Amplification products 336bp, 1177bp and 498 bp respectively were electophoresed in 2% agarose gel and visualised by ethidium bromide staining.
Results: Overall, of the 131 isolates strains *E. coli* 67(51%) carried pathogenic genes. From 67 strains 28(42%) were isolated from ambulance patients and 54 (80%) from women. The genes pap, hlyA and cnf occurred in several combinations. The combinations of pathogenic genes were as follow: 22 (33%) strains yielded pap gene, 2 (3%) hlyA, 9(13%) cnf1, 9 (13%) pap+cnf1, 8(12%) pap+hlyA, 3 (4%) cnf1+hlyA and 14 (20%) pap+cnf1+hlyA. From 22 strains pap+ and

14 strains (pap+cnf1+hlyA)+ 86% and 90% respectively were isolated from women.
Conclusion: Sixty seven (51%) *E. coli* isolates were found to carry at least one pathogenic gene. The most prevalent pathogenic gene was pap and combination pap+cnf1+hlyA. The frequency of pathogenic genes was higher in women, specifically in regard of strains with genes pap and pap+cnf1+hlyA.
The presence of pathogenic genes in *E. coli* contributes to colonisation, invasion, promote biofilm formation and persistence of strains in the urinary tract. Therefore knowledge of pathogenic genes of the microorganisms causing the UTI it would anticipate the evolution of infection in the host

P1296 Distribution of virulence factors in *E. coli* strains isolated from women with urinary tract infection by PCR

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Objective: *E. coli* is the most common cause of urinary tract infections (UTI). A variety of urovirulence factors are overrepresent in uropathogenic *E. coli*, including fimpriae and adhesins, cytotoxins such as a-haemolysin and cytotoxic necrotising factor 1, aerobacin-mediated iron uptake system and others. The dominance of strains expressing multiple urovirulence factors may further increase the risk for development of UTI specifically in women. In the present study, that was comparables in University hospital of Alexandroupolis in northern Greece, we analysed the distribution of genes pap, hlyA, cnf1, aer, sfa and afa in *E. coli* strains isolated from women with UTI.
Methods: A total of 103 strains *E. coli* were isolated from 103 women 18 to 83 years old with UTI. The laboratory criterion for acute *E. coli* UTI was the presence of positive culture on MC agar response with at least 105 CFU/ml. Multiplex PCR was performed to detect urovirulence genes pap, hlyA, cnf1, aer, sfa, afa in *E. coli* strains. Amplification products 336bp, 1177 bp, 498 bp, 602 bp, 410 bp and 750 bp respectively were electophoresed in 2% agarose gel and visualised by ethidium bromide staining.
Result: Seventy seven (75%) of the 103 strains *E. coli* were found to carry at least one urovirulence gene. Frequencies of genes pap, hlyA, CNF, aer sfa, afa in total 103 isolates were estimated as 45%, 20%, 25%, 47%, 27% and 6% respectively. The data indicated that 2%, 8%, 14%, 17%, 27% and 6% isolated carried six, five, four, three, two and one virulence factor respectively. However no virulence factors were found in 26 (25%) isolates.
Conclusion: The *E. coli* strains expressing multiple virulence factors comprise frequent cause developing UTI. The results showed that 75% of *E. coli* isolates carried at least one virulence factor. The occurrence of pap and aer was higher compared with the rest of others genes. In contrast, afa gene was carried in only 6 strains. Also in the present study was demonstrated that the multiplex PCR is an efficient tool for rapid detection and identification of UTI virulence factors in *E. coli* isolates.

P1297 Identification and genotyping of uropathogenic *Escherichia coli* strains isolated from patients with urinary tract infection based on papC and papG genes using PCR

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Introduction: *Escherichia coli* (*E. coli*) is the most common aetiologic agent of Urinary tract infections (UTIs). The most important virulence factor in Uropathogenic *E. coli* (UPEC) strains is P fimbriae. P fimbriae is coded by pap(pyelonephritis associated pili) operon. PapG is the most common adhesin in this fimbriae and papC is an important gene in this operon. Fimbriated uropathogenic strains of *E. coli* have an important role in causing UTI especially upper tract infections. In this study prevalence of pap operon (papC and papG genes) in *E. coli* strains isolated from nosocomial UTIs were evaluated.
Methods: A total of 182 *E. coli* isolates cultured from patients with different clinical forms of UTI referred to Shahre-kord teaching hospital were included. DNA of bacterial isolates was extracted by boiling method. PCR method was performed for presence of papC (pap operon) and papG genes. Also, genotyping of papG (pap operon) positive isolates were performed using multiplex PCR method.
Results: The prevalence of pap operon in isolates was 36.2%. Genotyping of P fimbriated isolates for papG gene by multiplex PCR method showed the prevalence of class 2 and class3 of papG gene, 23.1% and 6.6% respectively. None of the isolates had class 1 genotype. Furthermore, papG class 2 was predominant in patients with pyelonephritis and papG class 3 was predominant in ones with cystitis. In this study, no relationship between the presence of P fimbriae and sex of the patients was detected.
Conclusion: PapC (and papG)) genes have important roles in pathogenesis of fimbriated uropathogenic strains of *E. coli*. We recommend detection and genotyping of these genes in complicated UTIs in hospitalised patients enabling specific diagnosis and treatment of nosocomial infections with urinary tract origin.

P1298 Clonality and presence of fimbrial adhesin genes of *Escherichia coli* isolated in recurrent cystitis from a 48-year-old female

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Objectives: Urinary tract infections (UTI) are a common healthcare problem. Recurrent UTI in healthy, nonpregnant women is defined as three or more episodes of UTI during a 12-month period. It affects an estimated 20% of women at some time during their lifetimes. The uropathogenic *E. coli* (UPEC) possess adherence factors called pili or fimbriae (type 1 and P), which allow them to successfully initiate UTI infections. This adhesins are involved in the colonisation of mucosal cells, proliferation and cell damage, thus increasing the time of survival in the host. The aim of this study was to determine the occurrence of virulence genes and verify the clonal relation of *E. coli* isolates from recurrent cystitis.
Methods: A total of five *E. coli* isolates were recovered from a 48 year old female with recurrent urinary tract infection. Three strains were isolated from urine during six months and two strains were isolated from perineum and rectal swabs, respectively. Identification and antimicrobial susceptibility testing was determinate by VITEK 2 system. The presence of fimbrial adhesins was performed by PCR, using two pairs of primers: PAP1/PAP2 and fimH_F/fimH_R that amplify type P and type 1 fimbrial adhesins, respectively. Clonal relationship was assessed by M13 fingerprinting.
Results: The strains showed the same resistance profile and were resistant to trimethoprim/sulfamethoxazole, ciprofloxacin, norfloxacin, and showed susceptibility to cephalosporins (2nd and 3th generation), nitrofurantoin and fosfomycin. All strains exhibited the type 1 fimbrial adhesin and the same M13 fingerprint profile, including the strains identified from perineum and rectal swab. These results suggest that recurrent infections are due to the patient's inability to eliminate the microorganism (relapse), and not by reinfection, since all strains isolated in the period of 6 months belong to the same clone.
Conclusions: One of the main factors for the occurrence of urinary tract infections is the presence of adhesins, allowing the adherence of bacteria to uroepithelium hindering its removal, even after therapy.

P1299 Collagen-like proteins from the enterohaemorrhagic *E. coli* strain O157:H7

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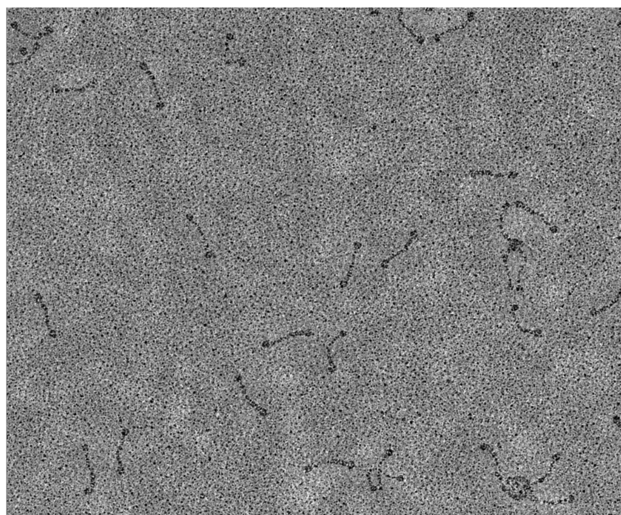
Many phage and bacterial genomes contain collagen-like sequences. The *Escherichia coli* strain O157:H7, a serious enterohaemorrhagic pathogen, has eight putative collagen-like genes that are missing in harmless *E. coli* strains. To help establish if collagen-like proteins contribute to O157:H7 virulence we have cloned one of these genes, EclA, into a laboratory strain and analysed it biophysically. Analytical ultracentrifugtion and

dynamic light-scattering showed that EclA is trimeric in solution, with a molecular weight of 132 kDa. EclA forms characteristic dumbbell-shaped structures visible in electron microscopy images. The "stalk" region adopts a collagen triple-helical conformation with a maximum ellipticity at 220 nm demonstrated by CD spectroscopy, while the CD of full-length EclA is dominated by alpha-helical structure localised to its N-terminal region. The thermal denaturation of the stalk showed a single transition at 42ºC, much higher than that of mammalian collagens, that was consistent with the loss of triple helical structure. On the other hand, full-length EclA showed two transitions: the first transition at approximately 42ºC corresponds to loss of triple-helical structure; the second one at 52ºC corresponds to loss of alpha-helical structure. The first transition is much higher than the equivalent seen for mammalian collagens. The results show that EclA behaves like an unexpectedly stable collagen protein. While the function of these proteins needs to be elucidated, mRNA transcripts of collagen-like genes were detected by RT-PCR both in stationary and exponentially growing cells, suggesting protein expression and functional significance.



P1300 **Intracellular crystallisation caused by *Proteus mirabilis* plays a significant role in recurrent urolithiasis**

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Objectives: *Proteus mirabilis* are the most common bacilli associated with the formation of bacteria-induced bladder and kidney stones. In 50% of cases, this is recurrent illness, which can lead to the loss of kidneys if not properly treated. Bacterial urease is the essential virulence factor in stones formation. Ammonia, produced by the enzymatic hydrolysis of urea, elevates urine pH causing a supersaturation and crystallisation of struvite and apatite. Crystals may grow intra as well as peribacterially. The aim of this work was to show that *P. mirabilis* bacilli can grow and form crystals inside urothelial cells. In this enviroment bacteria may be protected against antibiotic killing and thus cause persistent or recurrent infections.

Methods: *P. mirabilis* strains were recovered from encrusted biofilm on Foley catheter of long-term catheterised patients. The in vitro model was used to analyse intracellular growth and crystallisation. In this model human renal (HRPTEC), ureter (Hu 608) and bladder (HCV 29) epithelial cells were infected and incubated (3–72 h) in the presence of synthetic urine and amikacin to prevent extracellular bacterial growth. Number of intracellular bacteria was determined after host cells lysis by plating lysates on TSB plates. Crystallisation was determined using radioactive isotope of calcium. Crystals and bacteria were also observed by crystal violet and van Kossa method staining.

Results: It was found that *P. mirabilis* isolated from urinary catheter demonstrated a significant ability to adhere and penetrate normal urothelium, especially Hu 608 cell lines. These strains could multiply intracellularly within epithelial cells and were able to induce crystallisation. From 24 to 72 hours after infection a degree of crystallisation increased and it depended on the number of bacteria within host cells. This finding was confirmed by microscopy. The dark deposites of calcium salts within infected epithelial cells were observed.

Conclusion: Our results showed *P. mirabilis* ability to persist and form crystals inside the host cells, despite antibiotics presence. In this situation bacteria induce infection, usually chronic, which commonly results in the formation of bladder and kidney stones.

P1301 **Comparison of the invasiveness properties of Iranian *Shigella flexneri* wild type vs. mutants by cell culture and electron microscopy**

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Objectives: Among pathogenic microorganisms, invasive bacteria such as shigella has the ability to penetrate mammalian epithelial cells both in vivo and in vitro. Morona etal, produced a *S. flexneri* mutant and showed invasiveness of mutants decreased compared to wild types. So, the aim of this study was comparing the invasiveness properties of the *S. flexneri* wild types (icsA+ strains) v.s *S. flexneri* mutants (icsA defected) by cell culture and electron microscopy.

Methods: *Shigella flexneri* strains were isolated by standard bacterial methods from faecal specimens of children attending to the 3 children's hospitals of Tehran between January 2003 to December 2003. DNA isolation, was done by sodium perchlorate 4M method. Existence of icsA gene was determined by PCR. Mutation forming was done by allelic exchange and using pCACTUS-icsA:: amp r. HeLa cell culture were prepared using MEM medium and Fetal calf serum and infected with both *S. flexneri* wild types and mutants strains

Continuously, electron microscopy investigation was done after fixation, using resin spur and staining gold sections with saturated Uranyl acetate and Lead citrate.

Results: From 100 *S. flexneri* strains recovered from stool specimens, 46% were icsA + by PCR. All transformed bacteria were selected by temperature sensitivity and sucrase levan production and resistance to ampicillin. Further PCR on mutants showed no PCR product detection. Invasive shigella strains penetrated in to HeLa cells compare to mutants which may adhered but not entered. Further more, pseudopod structures used to facilitate bacterial cell-to-cell spread were readily identified by electron microscopy in wild types.

Conclusion: In this study, all clinical isolated *S. flexneri* wildtypes which were IcsA+, showed similar internalisation, cell existence and cell disruption. In addition, pseudopod filaments were confirmed with electron microscopy. In contrast, most of mutants icsA– could not enter to HeLa cells. Moreover, a few number of these bacteria could adhere and enter to the HeLa cells, but cell disruption was not detected. These bacteria might produce necrosis later, but not detected after 3 h incubation.

P1302 **GATC sites might regulate virulence of *Salmonella typhimurium***

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Background and Objectives: Pathogenesis of Salmonellosis depends upon a large number of factors controlled by an array of genes that synergise into the actual virulence of *Salmonella*. A study was undertaken to observe the effect of dam and/or seqA on the virulence and stress response of *Salmonella typhimurium*.

Methods: All experiments described in this paper were carried out with *Salmonella enterica* serovar Typhimurium strain SL1344, LT2, and their isogenic mutants dam– and seqA–, respectively. For the virulence test, we are used LD50, competitive index (CI) and bacterial enumeration from target organs such as liver and spleen. For the study of the effect of dam or seqA mutation on the stress response, we have chosen to

study response of these mutants toward bile, acidity, oxygen peroxide and antimicrobial peptides were tested.
Results: Our results show that dam or mutation attenuates *Salmonella* virulence either after intraperitoneal or oral infection. LD50, CI and enumeration of these mutants in liver and spleen prove a significantly decrease of virulence. On the other hand, in our work we are demonstrating that dam and seqA gene might control *Salmonella typhimurium* stress response (acid, bile, H2O2 and antimicrobial peptide).
Conclusion: Findings indicated that the dam and seqA genes may regulate virulence and stress response. We suggest that GATC distribution among DNA can regulate virulence and stress response of *Salmonella typhimurium*.

P1303 stj fimbrial operon has a role on *S. typhimurium* pathogenicity

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Objectives: To exhibit the role of stj fimbrial operon on the patogenicity of S. Typhimurium by animal experiments using mice model system.
Methods: The left and the right-end fragments of stjA gene were amplified with following primers; left-end F primer having BglII cutting site: GCAGATCTGCGGAAGTGATTTCACCGG/B primer having SalI cutting site CGGTCGACCCAAATGACATGTAAT-GCGCGGGTCGGG; right-end F primer having SalI cutting site: CGGTCGACCTCCAGCATTCACATGGAATATTCAACACC/B primer having XbaI cutting site GCTCTAGATCCAGTTTCACACCATTGTCG-GTC. Amplified fragments were than cloned to pGB704 vector plasmid. A kanamisin gene cassette was inserted between the two fragments on the vector plasmid. The recombinant plasmid was transformed to the S. Typhimurium IR 715 strain by conjugation. By the aid of left and the right-end fragments of stjA gene on the vector plasmid homolog recombination was occurred with the stjA gene on the chromosome of the host and the gene was knocked-out with kanamisin gene cassette. Animal experiments were performed with the mutant and wild type strain of S. Typhimurium.
Results: The experiments based on the competition between the wild type strain and degenerate mutant strain of stj operon. To construct the knocked-out mutant strain, one of the five genes in the operon, stjA, was degenerated with the kanamisin gene cassette on the plasmid pGP704. The mutants were named S. Typhimurium MA44 and used in animal experiment as knocked-out mutant strain of stjA gene. In the animal experiments, mice model system was used. AJB715 (S. Typhimurium lack of acid phosphotase activity) was used as the stjA wild type strain. 6–8 week female mice were inoculated orally with MA44 and AJB715. Viable cells in faeces and organ samples were counted and analysed statistically. It was observed that there was a significant ($p < 0.05$) reduction in the number of MA44 mutant strain in comparison to AJB715 wild type strain.
Conclusions: The results exhibited that stj operon plays an important role in the pathogenicity of S. Typhimurium by effecting the reproduction and stability functions of host organism in the target tissue systems.
Acknowledgements: This work was supported by the Scientific and Technological Research Council of Turkey (TUBITAK) grant 107T459.

P1304 Prevalence of virulence genes among nosocomial and cystic fibrosis isolates of *Pseudomonas aeruginosa* from Bulgaria

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Objectives: To study the prevalence of different virulence factors (adhesins, invasins and type III effector proteins) in clinical isolates of *Pseudomonas aeruginosa* and to perform a comparative analysis of their incidence according to the patients' population, localisation of infection and antimicrobial resistance of the strains.
Methods: A total of 202 clinical *P. aeruginosa* isolates obtained from in-patients and cystic fibrosis (CF) patients was collected during 2001–2006 from five university hospitals in Sofia, Bulgaria. Polymerase chain reaction amplification and sequencing were carried out to determine the distribution of the following virulence genes: algD (encoding alginate), pilB (type IV pili), nan1 (neuraminidase), lasB (elastase B), plcH (haemolytic phospholipase C), exoS (exoenzyme S) and exoU (exoenzyme U).
Results: The frequencies of virulence genes in all studied strains were: algD – 91.1%, pilB – 23.8%, nan1 – 21.3%, plcH – 91.6%, lasB – 100%, exoS – 62.4%, exoU – 30.2%. The prevalence of nan1 was higher in CF respiratory isolates (38.1%) than in non-CF isolates (16.9%) – $P < 0.01$. The distribution of pilB (9.5%) and plcH (71.4%) among CF isolates was lower than in non-CF nosocomial strains (respectively, 26.9% ($P < 0.01$) and 96.9% ($P < 0.001$)). The dissemination of virulence genes varied according to the localisation of nosocomial infections in non-CF patients. The relative proportion of multidrug-resistant (MDR) nosocomial strains of *P. aeruginosa* expressing a great number of virulence factors ($\geqslant 5$) (38.1%) was higher than the proportion of non-MDR isolates with a great number of virulence factors (17.6%) – $P < 0.01$.
Conclusions: The neuraminidase plays a key role in CF pulmonary disease evolution. Nonpilus adhesins such as the slime layer composed of alginate are the major virulence factors for attachment of *P. aeruginosa* to the respiratory epithelial cells in CF patients. Combined horizontal gene transfer and recombination of pathogenicity islands and mobile genetic elements containing antimicrobial resistance genes are the probable causes for selection of MDR *P. aeruginosa* strains with a large number of virulence factors.

P1305 Quorum sensing-deficient respiratory isolates of *Pseudomonas aeruginosa*

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Objective: *Pseudomonas aeruginosa* (PA) is an opportunistic pathogen which produces several virulence factors including elastase, alkaline protease, pyocyanin and rhamnolipid. The genes for these products are often controlled by quorum sensing (QS) systems. In PA, two QS system were identified, LasR/I and RhlR /I. We tried to analyse QS regulated virulence factors of respiratory PA isolates and determine QS deficient ones.
Methods: PA strains (n: 100) isolated from lower respiratory tract infections were used. Production of elastase, alkali protease, pyocyanin and biofilm were quantified spectrophotometrically. PA01 strain was used as a standard reference strain. Forward and reverse primers for LasI, LasR, RhlI, RhlR were used for polymerase chain reaction(PCR) amplification of QS genes. Evaluation of biofilm-forming ability and genetic typing was done by ERIC-PCR. Antimicrobial resistance was determined by agar diffusion.
Results: Production of biofilm was detected in 49 strains, whereas 45 strains elastase, 53 strains alkaline protease and 31 strains were pyocyanin positive. In 16 of the patients any of these factors were found to be positive. Virulence factor negative strains were not cumulated in a antibiotic susceptibility pattern or a genotypic subgroup. Four of the QS related genes were negative in only 3 of the 16 strains.
Conclusion: Depending on the infected tissue some virulence factors play more important roles than the others in the pathogenesis of PA infections. During the infection some virulence factors may compensate for the loss of any single virulence factor. The impact of spontaneous loss of several virulence factors on the colonisation and infection process is not well known. Although in 16 of our PA isolates none of the 4 virulence factors were detected phenotypically, genes responsible for the production for these factors were negative in only 3 of them suggesting low reliability of phenotypic tests. We can also conclude that PA strains that lack functional QS can still produce infection.

P1306 ***Stenotrophomonas maltophilia* isolated from patients affected by cystic fibrosis: genotyping analysis and molecular characterisation of virulence determinants**

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Objectives: In recent years *Stenotrophomonas maltophilia* has gained considerable attention being one of the most prominent emerging pathogens in the airways of CF patients. The role of *S. maltophilia* in the pathophysiology of the CF lung disease has not been clearly elucidated, but a progressive deterioration in pulmonary function has been observed, particularly in patients chronically colonised for longer periods. Despite its increasing role as a human pathogen, little is known about the bacterium, particularly with regard to putative virulence factors. Due to the remarkable diversity among isolates it is extremely important to acquire a better definition of bacterial clones or lineages able to cause disease in CF patients.
Methods: *S. maltophilia* clinical strains, isolated from CF patients at the "Bambin Gesù" Hospital in Rome (OBG collection), were analysed for genomic diversity by Pulse Field Gel electrophoresis (PFGE) and restriction length polymorphism of the gyrase B gene (gyrB RFLP). By Subtractive Suppression Hybridisation (SSH) genome analysis, we started to identify specific DNA regions selectively present in *S. maltophilia* OBGTC9, a persistent CF clinical isolate.
Results: While the gyrB RFLP analysis allows to group our *S. maltophilia* strains into few clusters, PFGE reveals a high genomic diversity despite their origin from a single hospital. As far as the identification of potential virulence factors is concerned, we have obtained a SSH library and we have identified some specific DNA fragments present in *S. maltophilia* OBGTC9. These genomic regions are not widespread among the *S. maltophilia* strains of the OBG collection, but rather associated only with two strains (OBGTC9, OBGTC10) able to establish persistent infections in CF patients.
Conclusions: We have analysed a group of *S. maltophilia* clinical strains, stemming from persistent or sporadic infections from respiratory secretions of CF patients, for genomic diversity and for the presence of virulence traits which may contribute to the pathogenicity process. PFGE reveals a high genomic diversity of *S. maltophilia* isolates despite their origin from a single hospital. Moreover, by SSH analysis we have identified specific DNA regions present only in two *S. maltophilia* strains (both persistent CF clinical isolates), which may be considered putative virulence factors in chronic infection.
This work is supported by the Italian Cystic Fibrosis Foundation (Grant 7/2007)

P1307 **Antibody response of cystic fibrosis patients with *S. maltophilia* infection/colonisation to the *S. maltophilia* fimbria SMF-1**

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Objectives: *S. maltophilia* is an opportunistic pathogen with controversial role in lung disease of cystic fibrosis (CF) patients. This bacterium expresses fimbria (SMF-1) that may be involved in bacterial adherence to epithelial cells (de Oliveira-Garcia et al, Cel. Microbiol., 5: 625–636, 2003). It was evaluated the antibody response of cystic fibrosis (CF) patients naturally infected/colonised with *S. maltophilia* against SMF-1.
Methods: Twenty-three *S. maltophilia* isolates were obtained from respiratory tract of 13 CF patients attending to Pulmonology Unit of Medical School of University of São Paulo, São Paulo, Brazil. *S. maltophilia* isolates were identified by classical phenotypic tests and by PCR and DNA sequencing in some cases. Whole cells were submitted to imunnoblottings using rabbit antiserum raised against SMF-1 to assess SMF-1 production. Also human sera obtained from CF patients colonised/infected with *S. maltophilia* were used against SMF-1 purified to verify the production of antibodies. It was used goat anti-human IgG and anti-rabbit IgG conjugated to horseradish peroxidase as secondary antibodies. Other Gram-negative bacteria and human sera obtained from patients without *S. maltophilia* infection/colonisation were used as negative control. SMF-1 purified and rabbit antiserum raised against SMF-1 were used as positive control.
Results: All *S. maltophilia* isolates produced SMF-1. All human sera tested reacted with SMF-1. Sera from 04 patients presented high title (1:500), and 02 of these patients had clinical or pulmonary function decline and sera from one patient presented title 1:2 500.
Conclusion: The presence of antibodies against fimbria is a marker for infection and production of these antigens in vivo. SMF-1 was produced in vivo during natural *S. maltophilia* infections/colonisations and elicited an immune response against it. This is the first description about the production of human antibodies against SMF-1.
Financial support: FAPESP (02/13111–0)

P1308 **First description of *Staphylococcus lugdunensis* internalisation into epithelial cells**

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Objectives: *Staphylococcus lugdunensis* belongs to coagulase-negative Staphylococci(CoNS) and can occasionally cause serious invasive infections such as osteomyelitis and infective endocarditis. *S. lugdunensis* accounts for 18% of infective endocarditis and 44% of native valve endocarditis caused by CoNS. *S. lugdunensis* expresses potential virulence factors, including synergistic toxins, haemolysins, extracellular enzymes, several adhesion factors, which mediate binding to collagen type I and IV, human IgG, fibronectin and fibrinogen. Furthermore, a fibrinogen-binding protein (Fbl), with a high homology to clumping factor A of *S. aureus* has been described.
We therefore sought to investigate internalisation of several *S. lugdunensis* blood culture isolates into different epithelial cell lines.
Methods: Internalisation into the human bladder carcinoma cell line 5637 and Hep2 cells were measured using a FACS-based method as described previously. The bacteria were stained with FITC and were incubated for one hour with the cells, gentamicin and lysostaphin was added for half an hour. The cells were washed and treated with trypsin to remove adhering bacteria and to detach the eukaryotic cells. For further discrimination, a gating strategy was used, because bacteria were smaller in size and FITC stained.
Results: The internalisation of several *S. lugdunensis* blood culture isolates was significantly increased in contrast to non-invasive *S. carnosus* TM 300. Internalisation ratio was slightly less compared to *S. aureus* Cowan I strain.
Conclusion: Our experiments demonstrate for the first time internalisation of *S. lugdunensis* clinical isolates into epithelial cells in a FACS invasion assay. These results were in line with the clinical suspected invasive ability of this pathogen. Internalisation into eukariotic cells could explain an important step of *S. lugdunensis* pathogenicity in serious infections e.g. endocarditis in analogy to mechanisms described for *S. aureus*.

P1309 **The signal peptide of Panton-Valentine leukocidine LukS component induces increased adhesion to extracellular matrix components in *Staphylococcus aureus***

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Staphylococcus aureus necrotising pneumonia is a highly severe disease due to *S. aureus* strains carrying the Panton Valentine leucocidin (PVL) genes (lukS-PV & lukF-PV) encoded on various bacteriophages (such as phiSLT). PVL is a pore forming toxin inducing polymorphonuclear cell death by necrosis (at high concentration, 200 nM) or by mitochondrial pathway induced-apoptosis (low concentration 5 nM). However, clinical PVL+ strains from necrotising pneumonia display additional properties such as increased adhesion to matrix molecules (type I and IV collagens and laminin) that could play a role in bacterial adhesion to damaged airway epithelium in the course of necrotising pneumonia.

To investigate the basis of this increased adhesion to matrix proteins we transduced phiSLT in various *S. aureus* clinical and laboratory strains, and tested these strains for adhesion to various matrix molecules and to matrigel® (a solubulised basement membrane preparation).
Transduction of phiSLT in different backgrounds produced an adhesion phenotype to collagen I and IV, laminin, elastin and matrigel®, similar to that observed with clinical PVL+ strains. In addition, independent expression of each of the PVL component in the laboratory strain RN6390 induced the same adhesion phenotype with an effect more marked for lukS-PV than for lukF-PV. Cloning and expression of various LukS-PV sub-fragment revealed that the 28 aa signal peptide of LukS-PV induced this adhesion phenotype. A synthetic LukS-PV signal peptide but not other unrelated signal peptide, produced the same adhesion phenotype in vitro. This study shows for the first time in bacteria the potential post-secretory function of a bacterial signal peptide. One hypothesis is currently investigated: the cleaved signal peptide could be released outside the cytoplasmic membrane and, after non-covalent linking to the peptidoglycan or teichoic acids, could function as an adhesin for the matrix proteins.

P1310 Extracellular adherence protein of *Staphylococcus aureus* in wound infections: surprising differences in transcription and expression in vivo and in vitro

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Objectives: The extracellular adhesion protein (Eap) of *S. aureus* belongs to a group of secreted proteins that are non-covalently bound to the cell surface and exerts versatile effects that fulfil prerequisites for chronic, persistent infections: Adhesion to extracellular matrix molecules, endothelial (EC) and other cells, reduced angiogenesis and EC proliferation, immunmodulation and delayed wound healing in mice. Although eap is prevalent in 100% of clinical isolates, only little is known whether and how eap is transcribed and expressed by clinical isolates under in vivo and in vitro conditions. Objectives of the study were the examination of the eap transcription in vivo from human wounds and in vitro under defined conditions as well as the corresponding protein expression.
Methods: RNA-Isolation: RNA was isolated using a modified Trizol protocol. Wound swabs were snap frozen in liquid nitrogen immediately.
Quantification of eap transcription: Quantification was done by RT-PCR using specific RNA standards for eap and gyrB.
Protein detection: Standard protocols for isolation of proteins from supernatants and cell-wall attached proteins were applied.
Results: Our analyses show that eap is transcribed by all clinical isolates in a strain- and growth phase-dependent manner. Under in vivo conditions, transcription of eap was found to differ with the degree of infection, with severe infections possessing the highest eap expression, which was up to 500-fold higher than under in vitro conditions. Strains causing wound infections seem to have the capability to transcribe eap on a higher level than strain Newman, so far known to be the highest Eap producer. While most of the synthesised Eap in Newman is found non-covalently bound to the cell surface, we were not able to detect significant amounts of Eap either in the cell surface fractions or the supernatants of the wound isolate cultures.
Conclusions: Our results prove that eap is transcribed by all clinical isolates tested. Interestingly, eap transcription seemed to correlate with the degree of infection, with eap being transcribed at a higher level under severe conditions than in superficial infections. Surprisingly, eap transcription of clinical isolates did not appear to correspond with protein production, indicating either differences in transcription- and translation efficiencies, or a rapid turn-over of Eap in the culture supernatants of these strains.

P1311 *Staphylococcus aureus* encoding both TSST-1 and PVL in the UK

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Objectives: TSST-1 and PVL are superantigenic, synergohymenotropic toxins that have been associated with toxic shock syndrome and necrotising pneumonia, respectively. These toxins are rarely reported in the same strain of *S. aureus*. We sought to determine the genetic diversity and clinical impact of such isolates across England and Wales during 2005–7.
Methods: *S. aureus* referred to the national Staphylococcus Reference Laboratory from 2005 to September 2007, and that tested positive by PCR for the presence of toxin genes including the lukSF-PV and tst were retained for study. Isolates were characterised by spa typing, SmaI PFGE and MLST, as appropriate. In addition: agr allotype, SCCmec type and ccr type were determined together with MICs of a wide range of antibiotics. Patient demographic and clinical data were retained for analysis.
Results: Five MRSA and 16 MSSA encoding TSST-1 and PVL were identified. These were geographically and temporally unrelated. The five MRSA were from four paediatric patients (3 male) and one 65 year old female. Two isolates were from abscesses, one each from a nasal swab and eye infection, and one of unknown source. The MRSA were SCCmecIV, related to known ST5, 22 and 30 isolates by PFGE and spa typing, and were resistant to up to three antibiotic classes, in addition to β-lactams. Six of 16 MSSA were from males and nine from females. Available data indicated the age range was <1 to 58y (mode = 0 y, median = 31 y). Three isolates were associated with pneumonia, 1 with bacteraemia, 1 with wound infection, 2 with abscesses, 1 was from pus and 5 were from screening swabs. The MSSA isolates were genetically diverse (including ST5, 22 and 30), 1 isolate was resistant to ciprofloxacin, 9 resistant to tetracycline and 2 were resistant to trimethoprim.
Conclusion: Diverse lineages of *S. aureus* encoding both TSST-1 and PVL have been identified sporadically in geographically distinct areas of the UK. Resistance to multiple antibiotics was noted in MRSA, but MSSA isolates remain largely susceptible. Like other PVL positive *S. aureus*, these strains have a predilection for the young with attendant impact on clinical management and empiric therapeutic strategies.

P1312 Acid tolerance of *Streptococcus anginosus*

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Objectives: Streptococci belonging to *Streptococcus anginosus* comprise a large proportion of the resident oral microflora. In recent years, however, it has emerged that *S. anginosus* infection could be closely related with oral, esophagus and gastric cancers. Since the surfaces of these organs are generally acidic conditions, the aciduricity, the ability to survive and grow under conditions of low pH, of *S. anginosus* may be associated with the upper gastrointestinal cancers. To elucidate the aciduricity and the acid tolerance mechanisms of the microbes, in this study, we examined the viabilities and growth of *S. anginosus* in acidic conditions, and the putative acid tolerance mechanisms, the ATPase and arginine deiminase (ADI) activities, were compared to those of other oral streptococci.
Methods: *S. anginosus* ATCC 10713 and the clinical isolates, and other oral streptococci, *S. mutans* ATCC 25175, CS. sanguinis ATCC 10556, CS. gordonii ATCC 10558, CS. mitis ATCC 9811, CS. oralis ATCC 10557 and *S. salivarius* ATCC 7073, were used. The aciduricity of the bacteria was assessed by the viabilities of preacidified bacteria and the growth in acidic conditions by means of the culture methods using Todd Hewitt agar. The ATPase and ADI activities were measured by the colorimetric methods on the permeabilised bacterial cells.
Results: Among the seven oral streptococcal species tested, *S. anginosus* and *S. mutans* showed markedly higher viabilities after the preacidification at pH 4.0 for 60 min, whereas the growth depression in acidic

conditions (pH 4.5–5.0) for *S. anginosus* was much lower than that for *S. mutans*. Both *S. anginosus* and *S. mutans* showed relatively higher ATPase activities than the other oral streptococci. However, the ADI activity was detected in *S. anginosus*, *S. sanguinis* and *S. gordonii*, but not in the other oral streptococci including *S. mutans*. Furthermore, the ADI activity in *S. anginosus* was the highest among the three.
Conclusion: Among oral streptococci, *S. anginosus* could have a significant aciduric property, which may be ascribable to their ATPase and ADI activities.

P1313 Investigation of affinity properties of receptor protein G of group G streptococci in different flow-through models using the approaches of affinity high-performance monolithic disk chromatography

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Objectives: The in vitro study of surface proteins of pathogenic microorganisms in model systems imitating its natural microsurrounding allows to estimate the role of receptors in pathogenesis of bacteria. The aim of the present study was to develop several flow-through models with cell-imitators, intact and inactivated cells of Group G streptococci immobilised on CIM® monolithic stationary phases (BIA Separations, Slovenia) and to investigate them using the approaches of affinity high performance monolithic disk chromatography (AHPMDC) taking as a reference the interaction between surface protein G and human IgG, thoroughly studied before [1].
Methods: We developed and investigated four models of surface of *Streptococcus*: 1) cell-imitators on the base of monodisperse polymethylmethacrylate (PMMA) microspheres (1 mkm in diameter) with covalently attached recombinant protein G; 2) cell-imitators on the base of monodisperse PMMA microspheres functionalised with recombinant protein G and dextran, imitating polysaccharidic microsurrounding of the receptor protein, 3) inactivated cells of Group G streptococci (strain 148); 4) intact cells of Group G streptococci (strain 148). All models were covalently immobilised on the surface of flow-through pores of CIM® disks in order to examine such an affinity parameter as dissociation constant (Kdiss) of the interaction between protein G and human IgG by means of AHPMDC.
Results: Cell-imitators as well as cells themselves were immobilised on the surface of absolutely flow-through pores of monolithic sorbents and did not block them. Such a design of models allowed to realise the processes of elution at high speed (2.5 ml/min) in comparison to traditional affinity chromatography where average speed is about 1–2 ml/h. Kdiss evaluated for all the models described above were of the same magnitude, ranging around 10–6–10–7 M. These results are in good agreement with previously received data [1] and indicate that streptococcal protein G has an extremely high affinity to human IgG that does not depend on its microsurrounding and is not influenced by covalent immobilisation.
Conclusion: The developed methodical approach seems to be an attractive tool to study the interactions between human proteins and microorganisms in order to gain improved knowledge about the pathogenesis of the last ones.
References.

Reference(s)

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P1314 Bacteriophage genes distribution among GAS belonging to different M serotypes

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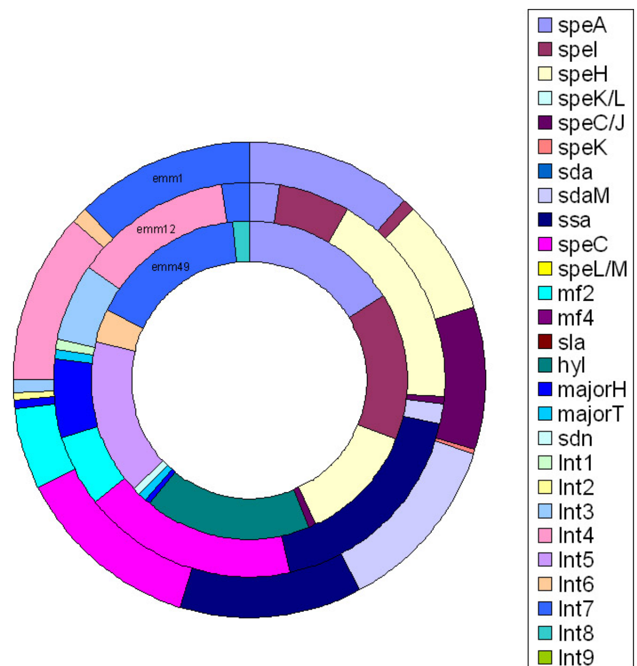
Objectives: In spite of the intense use of antibiotics in medical practice, the group A streptococcus (GAS) continues to be one of the most common bacterial pathogens of humans. Recent sequencing information has shown that the GAS possess tremendous genetic variability and one of the reasons for this is the presence of numerous streptococcal bacteriophages, which often carry genes encoding toxins, mitogens, enzymes and antibiotic resistance factors. Little is known about the real impact of specific phage or phage transported virulence genes in GAS pathogenicity. The aim of the present work was to study a possible correlation between the specific M serotype of GAS and bacteriophage content.
Methods: 75 GAS strains belonging to serotypes M1, M12 and M49 (31, 22 and 22) were studied. *Streptococcus pyogenes* strains type M1 and M12 were obtained from Chinese patients with scarlet fever in Beijing during 2007. M49 strains known to be from patients with invasive disease were obtained from CDC in the USA and collected between 2000–2005. Strains were analysed by PCR with a panel of 27 pairs of primers corresponding to the genes of phage related virulence factors and a set of nine phage related integrases. Results were substantiated by microarray hybridisation.
Results: 75 GAS strains belonging to 3 different M serotypes were analysed by PCR. Among the GAS strains from serotype M49, the highly prevalent genes found were speA, speI, speH, int5 and int7. In M1 strains speA, speH, speC, speC/J, ssa, sdaM, int4 and int7 were determined. In M12 strains the prevalent genes were speH, ssa, speC and int4 (fig1). A distinct correlation was found between the M serotype of GAS under study and phage gene content suggesting that temperate bacteriophages might be an important factors in the development of epidemic strains and may be involved in the triggering of GAS infections.



Phage genes distribution.

Conclusions: PCR analysis of bacteriophage gene content in the epidemic GAS reveals that strains belonging to the same serotype have similar bacteriophage gene patterns. The role of the bacteriophages in GAS pathogenicity is discussed.

P1315 Horizontal transfer of the *E. faecalis* pathogenicity island between *E. faecalis* and *E. faecium*

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Objectives: *E. faecalis* possesses a pathogenicity island (PAI) bearing the esp gene. In *E. faecium* a variant esp gene has been described and integration into a similar PAI structure is supposed. The data we

present here are to identify and characterise horizontal gene transfer of the *E. faecalis* PAI from *E. faecalis* to *E. faecium*.

Methods: Horizontal gene transfer was determined using a standard filter-mating procedure. Different donor and recipient stains (*E. faecium* 64/3; *E. faecalis* JH2–2, OG1RF) were tested. *E. faecium* transconjugants were checked for clonality with the recipient, i.a. by their PFGE patterns. Presence of esp was identified by PCR. PFGE, isolation of plasmid and genomic DNA were done according to standard procedures. Composition of the PAI was identified using single gene PCR, long PCR followed by restriction digestion and partly by DNA sequencing.

Results: While selecting for a transfer of erythromycin resistance between UW3114 and recipient 64/3 we were able to trigger parallel transfer of the esp gene. The donor and the esp-positive transconjugant possessed identical plasmids and plasmid digestion patterns suggesting chromosome to chromosome transfer of the esp/PAI from *E. faecalis* to *E. faecium*. We did PFGE with DNA from the donor, recipient, esp-positive and -negative transconjugants. Southern hybridisation identified a new 450 kb fragment possessing esp in the esp-positive transconjugant. Compared to the pattern of the recipient and the esp-negative transconjugant a 245 kb fragment disappeared in the PFGE pattern of the esp-positive transconjugant suggesting transfer of a ca. 205 (450–245) kb fragment bearing esp/PAI. Results of PCR and long PCR identified several deletions in the right end of the *E. faecalis* PAI in the donor and the esp-positive transconjugant.

Conclusions: In their original description, Shankar et al. (2002) were unable to transfer the newly described PAI in *E. faecalis*. However, by tagging the esp gene with a chloramphenicol marker Coburn et al. (2007) identified horizontal gene transfer of a 28 kb internal fragment of this PAI. We describe here for the first time inter-species transfer of the *E. faecalis* PAI into the genome of *E. faecium*. Our present data show transfer of a ca. 205 kb genomic fragment from *E. faecalis* bearing an incomplete but entire PAI element and integration of this fragment into the *E. faecium* genome.

P1316 Virulence factors and antimicrobial resistance in *H. pylori* clinical isolates by a multiple correspondence analysis

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The aim of this study was to determine the presence of different virulence factors and antimicrobial resistance in *H. pylori* clinical isolates and to analyse the results obtained by a multiple correspondence analysis (MCA).

Methods: 70 *H. pylori* strains were obtained from patients attending to the Gastroenterology Unit due to different symptomatology. 46 out of the 75 suffered of gastritis and 29 of peptic ulcer. Upper endoscopy was performed and biopsy cultured following standard methodology. DNA was extracted from a 48 h culture and PCR performed to detect cagA gene, vacA s1- and s2-alleles, babA2 gene, hpaA alleles and hopQ alleles. Specific primers for the cag empty site were also used to confirm the absence of the cagPAI (ES-cagA). All PCR products were analysed by agarose gel stained with ethidium bromide. Lewis antigen was determined by an Enzimoimmuno assay using monoclonal anti-LewisX and anti-LewisY and alcaline phosphatase labelled secondary antidodies. Metronidazole and clarithromycin resistance was determined by E-test.

Results: Three groups were detected by MCA: (1) strains positive for cagA gene (non discrepant with ES-cagA), vacAs1, hopQ type I-II, negative for babA2 and clarithromycin and metronidazole susceptible (group A). (2) Strains negative for cagA (discrepant with ES-cagA) and positive for vacAs2, hopQ type II, babA2, hpaA2, clarithromycin and metronidazole resistant and expressing Lewis antigen (LeXY or LeY) (group B). (3) A heterogeneous group (group C). Same results were detected when a dendogram for cluster analysis was drawn.

When the MCA analysis was performed in strains form ulcer patients the same 3 groups were detected with small differences. In strains from gastritis higher differences were found. cagA+, vacAs1, hpaA1 and hopQ I-II strains were associated with clarithromycin and metronidazole resistance (in ulcer strains were associated with susceptible ones), the presence of babA2, the expression of LewisXY antigen and the discrepancies with ES-cagA.

Conclusions: Strains with more virulence factors grouped together when MCA was performed. Moreover, in strains from gastritis virulence genes were associated with clarithromycin and metronidazole resistance whilst in ulcer strains they were associated with susceptible ones.

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P1317 Relevance of human beta defensin-1 in *Helicobacter pylori*-induced gastritis

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Objectives: Human beta defensins (HBDs) are antimicrobial peptides that are expressed by epithelia on mucosal surfaces. Recent reports have demonstrated that *Helicobacter pylori* infection induces HBD-2, whereas HBD-1 is thought to be expressed constitutively, and it's role in *H. pylori* infection is controversal. Therefore, the aim of the present study was to investigate the relevance of three SNPs of the HBD-1 gene in *H. pylori* induced chronic gastritis. We have also assessed mRNA expression of human beta-defensin 1 in by *H. pylori* infected AGS cells.

Methods: Three SNPs of the DEFB1 gene DEFB1 G-20A (rs11362), DEFB1 C-44G (rs1800972) and DEFB1 G-52A (rs1799946) were genotyped either by Custom TaqMan® SNP Genotyping Assays or by restriction fragment length polymorphisms (RFLP) in 150 patients with chronic active gastritis. Patients were examined by gastroduodenoscopy. HP positivity was detected by 13C-UBT and hystopathology. 100 serologically *H. pylori*-positive subjects without gastric or duodenal symptoms served as controls. Human beta-defensin1 mRNA expression in AGS cells was measured by quantitative RT-PCR reaction

Results: No significant difference was observed in the case of investigation the DEFB-1 SNPs at the region of −22 and −44. A definitive differences in the frequency of GA and AA genotypes of G-52 A SNP was observed between patients with gastritis and healthy controls. Conversely, the wild type genotype (GG) was significantly more frequent (47%) among healthy subjects than in patients (29%).

A dose dependent increase in HBD1 mRNA was observed in AGS cell line following infection with increasing number of *H. pylori*

Conclusion: We suppose, that the SNP in the −52 untranslated region of HBD-1 might be connected with a deficient function of human β defensin. This could lead to an increased colonisation of *H. pylori* in the stomac, with an ineffective clearence, and a consequent inflammation. Considering the results of the genetic and in vitro experiments our results draw the attention that not only the inducible, but also the constitutive form of human beta defensin have importance in pathogenesis of *H. pylori* induced gastritis.

P1318 Polymorphisms in the NOD1 and IL-8 gene, but not in the TLR4 gene are associated with *Helicobacter pylori*-induced duodenal ulcer and gastritis

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Objectives: Intracellular pathogen receptor NOD1 is involved in the epithelial cell sensing *Helicobacter pylori*, which results in a considerable interleukin (IL-8) production. The aim of this study was to evaluate the relationship between NOD1 and IL-8 genetic polymorphisms and the development of *H. pylori*-induced gastritis and duodenal ulcer (DU), as compared with TLR4 polymorphisms.

Methods: Eighty-five patients with DU and 135 patients with gastritis were enrolled in the study. Patients were examined by gastroduodenoscopy. HP positivity was detected by 13C-UBT and hystopathology. Seventy-five serologically *H. pylori*-positive subjects without gastric or duodenal symptoms served as controls. The G796A (E266K) NOD1 polymorphism was determined by restriction fragment length polymorphism, and the −251 IL-8 polymorphism by amplification

refractory mutation system method. The TLR4 (ASP/299/Gly and Thr/399/Ile) gene polymorphisms were examined by melting point analysis.

Results: AA homozygote mutant variants of NOD1 were detected in 20% of the *H. pylori*-positive patients with DU versus 7% of *H. pylori*-positive patients with gastritis and versus 6% of the *H. pylori*-positive healthy controls. The IL-8 heterozygote mutant variant was detected with a significantly higher frequency among the DU patients (57.6%) and those with gastritis (55.9%) than among the *H. pylori*-positive controls (40%). However, no significant correlation concerning the frequency of the TLR4 gene polymorphism could be revealed between any group of patients and the controls.

Conclusions: E266K CARD4/NOD1, but not the TLR4 gene polymorphism increases the risk of peptic ulceration in *H. pylori*-positive patients. The −251 IL-8 polymorphism was significantly associated with either gastritis or DU in *H. pylori*-infected subjects. Host factors including intracellular pathogen receptors and IL-8 production play an important role in *H. pylori*-induced gastric mucosal damage.

P1319 Intestinal Oxalobacter formigenes colonisation in children with calcium oxalate urolithiasis. A study from southeastern Poland.

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Objective: Oxalobacter formigenes is an obligate anaerobic Gram-negative bacterium colonising the colon of vertebrates, including humans. This organism utilises oxalate as the only source of carbon and therefore participates in oxalic acid homeostasis. In adults, it was suggested that the lack of colonisation with O. formigenes may lead to the intestinal oxalate hyperabsorption and to calcium oxalate urolithiasis (CaOxU) as a consequence. To our knowledge this problem was not investigated in paediatric population. Hence, the aim of our study was the assessment of O. formigenes colonisation rate in children with CaOxU.

Methods: DNA was extracted from the frozen faecal samples of 84 children and adolescents (41 boys, 43 girls) aged 4.9–17.6 years with CaOxU and 63 healthy, age- and gender matched subjects. Identification of O. formigenes was performed by polymerase chain reaction (PCR) using genus specific primers, according to Sidhu et al. (JCM 1999, 37, 1503–1509).

Results: O. formigenes was found in 23.8% (20/84) of patients with CaOxU and in 20.6% (13/63) of healthy controls.

Conclusions: In contrast to adults, we did not find any significant difference in O. formigenes colonisation rate between individuals with or without urolithiasis. However, an absence of O. formigenes in majority of children from both groups requires further explanation. It could be caused by an overuse of antibiotics in our region. Also, we can not exclude that extreme variability in the O. formigenes content of stool samples among individuals may be responsible for the low rate of O. formigenes identification in both examined groups.

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Antibacterial vaccines

P1320 Analysis of *Haemophilus influenzae* isolated from invasive disease, in Portugal, during extensive vaccination against *H. influenzae* serotype b

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Background: *Haemophilus influenzae* (Hi), an exclusively human pathogen is responsible for potentially life threatening invasive disease such as meningitis and septicaemia. Hi pathogenicity varies depending on the presence or absence of a capsule and the specific capsule type. Hi serotype b (Hib) vaccine was adopted in Portugal since 1994 and obligated since the year 2000 to children less than five years old. In vaccine era, non capsulated (NC) isolates have become the most common cause of invasive Hi disease in all age groups.

Objectives: We pretend to analyse Hi serotypes as well as antimicrobial susceptibility changes in the vaccine era and compare with previous studies in Portugal.

Methods: We collected 102 invasive isolates (20 CSF, 74 blood cultures and 8 pleural fluids), from 2000 to 2007, in Antibiotic Resistance Unit from the National Institute of Health, from several Hospital Laboratories in different regions of Portugal.

Identification of Hi was confirmed by standard procedures. Nitrocefin was used to determine β-lactamase production. Susceptibility testing was performed by micro dilution, according to the CLSI guidelines. Serotyping was performed by PCR.

Results: Strains were distributed by age as follow: forty-one strains (40.2%) were from children (<18 years old), being twenty eight of these (68.3%) from under 5 years old; fifty-nine strains (57.8%) were isolated from adults.

From all the invasive strains, 10.8% were β-lactamase producers and 5.5% with MIC values to ampicillin and clavulanic acid of 2 mg/L, were considered ampicillin-resistance-non-β-lactamase-producers (ARNBLP). Resistance to SXT was high: 19%.

As we expected, most strains (76.5%) were NC, being 15 of serotype b (15.3%).

Two of the 3 serotype a strains, were isolated from children. All 4 serotype f strains and 1 serotype d were from adults.

In a previous study of invasive strains, performed in our laboratory with strains isolated between 1989 and 2001, we observed a decline in serotype b strains, from 81% to 16%, accompanied by an increase of NC strains, from 19% to 80%.

Conclusions: This study continues to documents changes in invasive infections following the introduction of the vaccine in Portugal. We are assisting to an increment of NC strains as well as a great decline of Hi serotype b.

We also expect an increase of non-b-type strains in Hi invasive disease and a new rule in the virulence of these strains. So, it's imperative to continue this kind of studies.

P1321 Changes in invasive pneumococcal disease among children after the introduction of the heptavalent pneumococcal conjugate vaccine

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Objectives: Pneumococcal conjugate vaccine (PVC-7) has been reported to prevent invasive disease (IPD) by covering the most prevalent serotypes isolated from blood or CSF. Implementation of PCV-7 could have changed epidemiology, clinical and analytical features of IPD. We analysed the impact of PCV-7 in our community, focusing on emergence of nonvaccine serogrups, clinical diagnosis, incidence of empyema and inflammatory response (C-reactive protein [CRP], procalcitonin [PCT]).

Methods: we analysed 75 episodes of bacteraemia within two periods, from 1995 to 2001 (prelicensure period) and from 2002 to September 2007 (postlicensure) in children below 18 years old admitted in the Department of Paediatrics of a tertiary-care Hospital of Barcelona.

Results: A total of 76 children with pneumococcal bacteraemia were identified, with 28 episodes occurring in the prelicensure period and 48 in the postlicensure period. Forty-two (42.1%) were <2 years old, 39.5% were between 2 and 5 years old and 18.4% were >5 years old. Respiratory (60.5%), occult bacteraemia (15.8%), sepsis without focus (11.8%) and meningitis (7.9%) were the most frequent clinical manifestations, without statistical differences between the two periods. There was a trend towards increasing frequency of respiratory focus during the second period but changes in incidence of non-vaccine serotypes were not statistically significant. Serotypes 1 and 14 accounted for 44% of all isolates vs 29% in the pre-licensure period. Serotype 1 was significantly associated with empyema, increased CRP and PCT values, and length of hospitalisation (14±4 days). Serotypes 1 (4 cases), 15B, 19A and 24 caused IPD in 7 vaccinated children. High resistant

penicillin strains showed an increase from 17.9% to 25% and eritromycin from 21.4% to 31.3%, between the pre and postlicensure period.
Conclusions: We found an increased number of empyema cases caused by serotype 1, with high CRP and PCT during the postlicensure period. Changes towards the emergence of non-vaccine serotypes causing IPD are not significant in our area. We didn't find vaccinated children with IPD caused by serotypes included in PCV-7.

P1322 Knowledge of healthcare workers regarding occupational vaccinations

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Immunisation against diphteria, tetanus, poliomyelitis, hepatitis B and tuberculosis are mandatory for healthcare workers (HCW) in France. The Committee for Public Health (Haut Conseil de la Santé Publique) also strongly recommends immunisations against influenza, pertussis, varicella, measles and for non immunised HCW.
Objectives: To evaluate the knowledge of hospital HCW regarding mandatory or recommended vaccinations.
Methods: We performed a cross-sectional survey in two medical departments: one in an internal medicine department caring for immunosuppressed patients, and one in a paediatric department. A self-administered questionnaire was used to assess all the medical and non medical staff (physicians, students, nurses, paramedics and staff not caring for patients) knowledge regarding mandatory and recommended vaccinations, their current vaccination status and potential reasons for refusal of vaccine uptake.
Results: Three hundred and ninety-five questionnaires were analysed (280 from the Internal medicine and 115 from paediatric departments). Although 95% of the staff declared a good knowledge of the compulsory vaccination, only 20.7% of the internal medicine staff and 30.6% of the paediatric staff could cite them correctly ($p=0.05$). More than 70% declared knowing the recommended vaccinations: 68% cited influenza, 15.4%, pertussis, 5.7% measlesand 4.7%, varicella.
The overall influenza vaccination rate in 2006 was higher in paediatrics than in internal medicine unit (36% vs. 28%; $p=0.012$); 50% of the physicians, 42.7% of the students and 22.6% of the nursing staff received influenza vaccine. The strongest motivations for having received influenza vaccination were to protect patients (73%) and to protect oneself (67%). Contrarily, the main reasons for vaccination refusal were believing the vaccine to be inefficacious (53%) and fearing adverse effects (21%). Reasons for not having been vaccined against influenza may differ between nursing/paramedical staff and physician staff.
Conclusion: Knowledge about vaccinations is poor among HCW. Despite recommendations for annual influenza vaccination, the proportion of HCW vaccinated is still insufficient, mainly due to a misperception of the efficacy of the influenza vaccination. This study highlights the need to strengthen continuous education of HCW regarding vaccinations.

P1323 Serotype distribution of *Haemophilus influenzae* isolates from post-vaccinated Mexican children: comparison between antisera and PCR-based typing methods

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Objective: The world wide use of *Haemophilus influenzae* (Hi) type b conjugate vaccine has resulted in a significant decline in the incidence of invasive disease in children <5 years of age. The 3 doses scheme of polyribosyl ribitol phosphate (PRP)-T was licensed in our country in 1999. However it has been speculated that this may lead to a greater prevalence of nonencapsulated (NE) and encapsulated non-b strains. Surveillance studies are increasingly important in Mexico for monitoring the emergence of such strains. In this study we compare the serotype distribution of post-vaccine Hi isolates using PCR-based serotyping method to that obtained by slide agglutination serotyping (SAS), currently considered as the gold standard.
Methods: One hundred and eleven Hi strains were screened by the SAS method using polyvalent and monovalent antisera. A PCR assay for the bexA locus was used to detect encapsulated strains. The encapsulated isolates were genotyped using serotype-specific primers for the a, b, e, and f Hi types.
Results: In this study, the SAS method revealed 88 (79.3%) NE Hi strains, of which 6 were found to be types a (1), f (4) and e (1) when the PCR capsule genotyping was performed. Additional discrepancies were observed when 7 NE isolates detected by the PCR method were misidentified as serotypes b (6) and f (1) by SAS. The overall 7.2% (8/111) of the strains contained the f serotype-specific sequence. There was 11.7% discrepancy between the SAS and the PCR-based genotyping methods.
Conclusions: The data showed that Hi serotype f is circulating among post-vaccinated Mexican children. The gold standard method for serotyping the Hi strains still offers equivocal results. The genotyping PCR assay provides a more accurate alternative for determining Hi serotypes.

P1324 Immunological responses to BCG vaccination in *Mycobacterium bovis*-challenged cattle

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Objectives: BCG vaccination studies in cattle have shown extreme variability in protection against *Mycobacterium bovis* infection. A critical first step in the development of an improved vaccination is the understanding of the host immune response to infection and vaccination.
Methods: This study focused on four experimental cattle groups BCG Pasteur vaccinated *M. bovis* challenged, BCG vaccinated non challenged, non vaccinated *M. bovis* challenged and non vaccinated non challenged. mRNA expression levels of IFN gamma, TNF alpha, IL10 and IL4 were measured within thoracic lymph nodes of each animal using quantitative real time PCR. Protein levels of IFN gamma and TNF alpha were measured within each lymph node using immunohistochemistry.
Results: Challenge with *M. bovis* significantly increased IFN gamma mRNA levels (94%), reduced IL10 mRNA levels (56%) and suppressed IL4 mRNA expression completely. There was a significant increase in IFN gamma protein levels (on a score scale of 1–4, challenged animals averaged at 2.75 compared to non-challenged at 1.3) however TNF alpha mRNA and protein levels were not significantly affected by *M. bovis* challenge. BCG vaccination prior to challenge reduced the level of IFN gamma mRNA (81%) and IFN gamma protein (averaged score of 1.5) as compared to the non vaccinated challenged cattle. BCG vaccination without challenge appeared to have no effect on the cytokine and protein levels however IL4 mRNA was detectable. There was a strong positive correlation between IFN gamma mRNA and IFN gamma protein levels but no correlation was observed between TNF alpha mRNA and TNF alpha protein.
Conclusion: *M. bovis* infection activates a strong cell mediated immune response signified by the dramatic increase in IFN gamma mRNA expression. Subsequently IFN gamma protein levels increased leading to a strong T helper (TH) class 1 profile with the related reduction of IL10 and IL4 mRNA expression.
Cattle vaccinated with BCG before challenge showed an increase in IFN gamma mRNA expression but to a lesser degree as compared to non vaccinated animals due to the primed immune response. The IL10 mRNA levels were not significantly reduced in vaccinated animals and so dampened the pro-inflammatory response producing a lower level of pathology within the vaccinated animals. Finally, it appears that IL10 mRNA may experience post transcriptional regulation due to the absence of a correlation between mRNA and protein levels.

P1325 REPEVAX® can be safely administered as a booster one month after tetanus, diphtheria and poliomyelitis vaccination: an option for managing increasing incidence of Bordetella pertussis (whooping cough) infection

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Objectives: In France it is recommended to administer a booster dose of a pertussis-containing vaccine in adults which can result in them receiving 2 doses of tetanus (T) and/or diphtheria (D/d) and/or poliomyelitis (inactivated, IPV) containing vaccine within a short period (eg catch-up programme or pertussis outbreak). In children and teenagers it has been shown that boosters can be safely given with intervals as short as about 2 years, but in adults there are no data for intervals less than 5 years. We assessed the safety of a booster dose of a pertussis-containing vaccine (acellular component, aP) shortly after a Td-IPV booster.
Methods: 500 healthy adults 18–40 years, having received ⩾5 doses of T, D and poliomyelitis vaccine by the age of 18, with the last dose of T and/or D and/or poliomyelitis vaccine given alone or combined at least 5 years previously, were randomised to receive either Td-IPV (REVAXIS®; Group 1) or placebo (Group 2) followed by Td5aP-IPV (REPEVAX®) in both groups one month later in a double-blind trial. Adverse events, including pre-listed events for the first 7 days (injection-site erythema, swelling, and pain, oral temperature ⩾37.5°C, headache and myalgia) were recorded on a diary card.
Results: The groups were similar in terms of age, sex ratio, weight, height, number of previous vaccinations with D, T, and poliomyelitis and intervals between last D, T or poliomyelitis vaccination and study start. 484 subjects who received the second injection were included in the REPEVAX® safety analysis. The adverse event rates reported after REPEVAX® were similar in both groups except for a lower incidence of solicited injection-site reactions in subjects in Group 1.
In Group 1 the rates of injection-site swelling and pain reported within 7 days after REPEVAX® were lower than in Group 2 (19.4% vs 27.7% and 82.6% vs 92.1%, respectively). The confidence interval of the difference between groups did not include 0 (swelling: –8.3 [–15.7; –0.7], pain: –9.5 [–15.5; –3.6]), but this was not judged to be clinically meaningful.

Adverse events	Group 1, % (n = 242)	Group 2, % (n = 242)
Pre-listed (Day 0–7)		
Injection-site reactions	84.3	93.0
Systemic adverse events	36.0	37.6
Spontaneously reported (Day 0–14)		
Injection-site reactions	14.9	16.1
Systemic adverse events	20.2	19.0

Conclusion: These data support the use of REPEVAX® in adults, even one month after a booster with Td-IPV. REPEVAX® can be used as a booster to reduce the incidence of whooping cough.

P1326 Elaboration of a type three secretion system-based *Pseudomonas aeruginosa* live vector for immunotherapy purposes

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Objectives: Numerous immunotherapy methods have been designed to eliminate cell populations (infected or tumour cells). To trigger an efficient cytotoxic T lymphocyte (CTL)-based response, the target antigen (Ag) (i.e., viral protein) must 1) be delivered to dendritic cell (DC) in order to obtain an MHC-I restricted presentation and a subsequent CTL activation, and 2) be associated with a danger signal in order to activate DC. We took advantage of *Pseudomonas aeruginosa* type three secretion system (TTSS) to elaborate a bacterial immunotherapy live vector. TTSS is used by Gram-negative rods to inject protein effectors inside target cells such as (for *P. aeruginosa*) dendritic cells and PMN. A TTSS-based live vector would ensure both danger signal and delivery of Ag to DC.
Methods and Results: By fusing different eukaryotic proteins with a non-toxic fragment of a TTSS effector, we obtained the secretion by *P. aeruginosa* TTSS of these fusion proteins. Ovalbumin was first used as a model antigen. In vitro, murine dendritic cells having incubated with the ovalbumin-delivering vector were able to activate specific anti-ovalbumin CTL. In vivo, after 2 injections of 5.10e6 ovalbumin-delivering bacteria, mice generated anti-ovalbumin CTL. The efficiency of this response was assessed by injecting ovalbumin-expressing syngenic melanoma cells to these mice; we obtain a complete protection (no tumour onset) of 7/8 animals, but no protection in animals injected with a vector delivering a control Ag. A significant but less vigorous protection was observed when the vector delivered a native Ag of melanoma.
The bacteria had been already attenuated by deleting the genes of TTSS effectors ExoS and ExoT. Virulence was further altered by deleting central genes of quorum sensing (coding for synthases LasI and RhlI) and/or gene aroA (rendering the bacteria auxotrophic for aromatic amino-acids). The maximally attenuated vector had a LD50 more than 100 times higher and a conserved efficiency as immunotherapy vector.
We explored another model by vectorising *Plasmodium yoelii* circumsporozoite protein as to target infection at the liver stage. We observed a significantly delayed blood smear positivation after sporozoite infection in vaccinated mouse, indicating that the immune response triggered by the vector was partly efficient.
Conclusion: We believe that this TTSS-based *P. aeruginosa* vector is a promising immunotherapy tool in both infectious and tumour diseases.

P1327 The application of *Neisseria meningitidis* serogroup B outer membrane vesicle as an adjuvant to induce specific antibody against the LPS of *Brucella abortus* S-99 in animal model

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Objective: Smoth lipopolysaccharide(S-LPS)of *Brucella abortus* can be candidated as a potent human vaccine. The adjuvants with microbial origins are more considerable among the other current adjuvants nowadays. The outer membrane vesicle(OMV)of *Neisseria meningitidis* serogroup B is one of the newly studied adjuvants with microbial origin.
Methods: The S-LPS of *Brucella* was extracted by Hot phenol-water with some modification as described previously. The noncovalent complex of S-LPS-OMV was injected subcutaneously into groups of ten mice with boosters on days 14 and 28 after the primary immunisation. The following groups were used as control: 1-LPS, 2-LPS plus incomplete Freunds adjuvan(IFA)and 3-LPS plus Freund's complete adjuvant (CFA). The mice were bled on days 0, 14, 28, 42.The humoral immune responses evaluated by detection of specific anti-LPS IgM and IgG titre via ELISA method.
Results: Anti-LPS IgG titre(demonstrated by ELISA)for LPS plus OMV has been increased 3.9, 3.18 and 1.58 fold after 14 days in comparison with the LPS, LPS plus IFA and LPS plus CFA control group. The application of LPS plus CFA led to higher level anti-LPS IgG titre than LPS plus IFA and LPS with 1.98 and 2.5 fold, respectively. The anti-LPS IgG titre in LPS plus IFA control group was not significantly increased in comparison with the LPS group. Booster doses have been effective to significantly increase anti-LPS IgG titre in all groups after 28 days. An increase in anti-LPS IgG titre following the second and third dose of LPS plus OMV induced 6 and 12.4 fold titres in comparison with the first dose. The highest anti-LPS IgG titre detected two weeks after the third dose(after 42 days)of LPS plus OMV.Comparative study of anti-LPS IgM titres were not revealed any significant level in all groups.

Conclusion: In this study, we showed that *N. meningitidis* serogroup B OMV in noncovalent complex with *Brucella abortus* S99 LPS induces high level of anti-LPS IgG and our purified OMV could act as an effective adjuvant. Since the application of most adjuvants is restricted because of hypersensitivity reactions and the safety of OMV in humans has been demonstrated in many studies, OMV would be a safe adjuvant which does not cause hypersensitivity and DTH reactions. Therefore noncovalent complex of OMV-*B. abortus* LPS may be a candidate for further development as a brucellosis vaccine for human use.

P1328 Immunological evaluation of vi-vapsular polysaccharide of *Salmonella typhi* vaccine by serum bactericidal assay

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Objectives: *Salmonella typhi* Vi-capsular polysaccharide (Vi-CPS) is an essential virulence factor and a safe and reliable vaccine against typhoid fever (Acharya et al, 1975: Szu et al, 1994: Yang et al, 2001). Since there is no any animal model for *Salmonella typhi* fever to evaluate protective efficacy of Vi-CPS vaccine, we recommend, serum bactericidal assay (SBA) as a golden standard to evaluate its potency.
Methods: Vi-CPS was extracted from *Salmonella typhi* Ty6S (CSBPI-B-191) according to the modified Gotschlich method (Ahmadi et al, 1999). Fifty μg of purified Vi-CPS was injected intramuscularly into three groups of five rabbits, Groups 2 and 3 were received additional booster dose of 50 μg Vi-CPS on day 15 and days 15 and 30 respectively. Each group of pooled serum was tested by SBA on days 0, 15, 30 and 45.
Results: The titers of SBA of anti Vi-CPS were 4, 16 and 16 for group 1 and 4, 32 and 32 for group 2 and 16, 64 and 64 for group 3 in days 15, 30 and 45 respectively.
Our investigation reveals that; Vi-CPS is a potent immunogen. Because, at even one does of 50 μg, could show an excellent bactericidal effect against pathogenic *Salmonella typhi*.
Conclusions: Though Vi-CPS is a reliable vaccine against typhoid fever but sometimes depolymerisation take place during purification and may cause low potency. This draw back can be resolved through potency test. Since passive hemaglutination test recommended by WHO., does not indicate potency of the vaccine, we recommend SBA method to evaluate the ability of Vi-CPS to kill pathogenic *Salmonella typhi*, as it has been shown in our present results.

P1329 Constraction of polyvalent anti-streptococcal vaccines and analysis of their polyvalent features

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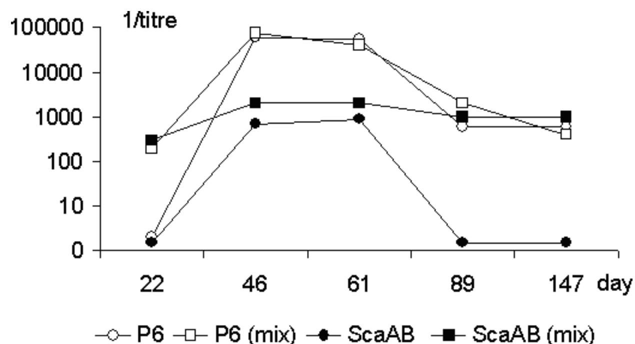
Objectives: In many occasions when antibiotic prophylactics fails vaccination becomes the matter of choice. More studies have been made for streptococcal vaccine development. The most common approach for streptococcal vaccine generation is based on the obtaining the conjugates between the polyvalent protein antigens and polysaccharide capsule. This strategy provides both an immunogenicity, with significant protection and broad level of type specificity. The present work presents data on comparison of two variants of vaccines: one based on mixture of recombinant proteins and another on the conjugation of type III GBS (PS) capsular polysaccharide with GBS recombinant cell surface polypeptides.
Methods: Conjugation of polypeptides with PS was obtained by reductive amination. Immune response had been studied in mice (double immunisation with aluminum hydroxide as adjuvant). Antibody titer was tested by ELISA. Biological activity of antibodies was tested by opsonophagocytosis employing mice peritoneal macrophages.
Results: After optimisation of PS-peptides conjugation conditions (optimal concentration of the components, oxidation time, the temperature of reaction etc.) the best chemical reaction conditions for two of the recombinant polypeptides under study (P6 and ScaAB) were found. Vaccine preparations containing P6-PS or ScaAB-PS reacted with antibodies against each of the vaccine components which proved that the antigenic structure had been preserved. Covalent binding of the PS to P6 or ScaAB lead to the four fold increase of the immune response relatively mono peptides usage with maximum titers against P6 and ScaAB 1/13000 or 1/104000 concordantly. PS after the binding also was immunogenic which was proved by appearance of specific IgG which were detected up till 70 days after the immunisation. In case of the mixture of the peptides P6 and ScaAB the titer of specific antibodies against ScaAB-components was also increased as well as length of the antibody circulation – 150 days. Mice antibodies obtained after immunisation with both type of vaccines were active against GBS belonging to different serotypes.



P6- and ScaAB-specific immune response (mixt vaccine and free form immunisation).

Conclusion: Usage of polyvalent vaccine preparations based on cell surface expressed recombinant polypeptides of GBS can dramatically increase their immunological features relatively to monovaccines.

P1330 TSA gene of *Leishmania major* cloned in eukaryotic expression vector could stimulated immune system in mice

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Objectives: TSA (thiol-specific-antioxidant antigen) is the immunodominant antigen of *Leishmania major* considered as the most promising molecule for a recombinant or DNA vaccine candidate against leishmaniasis. In the present work.
Methods: Genomic DNA of TSA protein was extracted and amplificated as a template. The PCR product has cloned into pTZ57R/T vector followed by subcloning into the eukaryotic expression vector pcDNA3 (EcoRI and HindIII sites). The expression of plasmid containing TSA gene was demonstrated by SDS-PAGE and Western-blot. These results indicated successful expression of plasmid containing TSA gene in eukaryotic cells.
Results: In the present study, we evaluated TSA-encoded DNA vaccine against *L. major* in BALB/C mice. IgG and IFN-g values were markedly increased in the immunised group, which were significantly higher than in the control groups ($p < 0.05$) following immunisation and after challenge with *Leishmania major*. IL-4 values were increased in all groups, but there was no statistical difference between the groups ($p > 0.05$) following immunisation and after challenge with *Leishmania major*.
Conclusion: The findings of this study indicated that the TSA-encoded DNA vaccine increased the cellular response and induced protection against infection with *Leishmania* in the mice. The TSA-encoded DNA vaccine may be an excellent candidate for future vaccine developments against *Leishmania*.

P1331 Diversity of pneumococcal surface protein A among prevalent clones causing invasive disease in adult patients in Barcelona (1997–2006)

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Objectives: Pneumococcal surface protein A (PspA) is a recognised major virulence factor and it has been classified into family 1 (Fam1: clades 1 and 2), family 2 (Fam2: clades 3, 4 and 5), and family 3 (Fam3: clade 6). After PCV7 vaccination, serotype/clone replacement has been observed worldwide. The aim of this study was to determine the distribution of Fam and clade of PspA among invasive representative *S. pneumoniae* (Spn) strains from adult patients from the Hospital Universitari de Bellvitge, Barcelona, in order to establish the possible use of PspA as a vaccine candidate.
Methods: In our Hospital, 90% (n = 395) of the invasive pneumococci isolated from adult patients from 1997 to 2006 were studied by PFGE (SmaI) and serotyping. A total of 72% of the studied strains were grouped into 28 major PFGE patterns. A selection of 49 invasive pneumococci, representative of those dominant PFGE patterns, was characterised by MLST (ST) and PspA. Clade-defining region of PspA was amplified by PCR, sequenced and compared with published data.
Results: PspA sequence analysis revealed that 30 strains belonged to Fam2, 18 strains to Fam1 and one strain was non-typeable. No Fam3 strains were detected. Association between serotypes and Fam/clade was not found, however a relationship between Fam/clade and ST was observed:
– Fam1/clade1 (n = 37) was associated with Spain6B-2, Spain14–5, England14–9, Colombia5–19, Sweden1–28, Sweden1–40, Netherlands8–33, ST97-serotype 10, ST1026-serotype 20, ST311-serotype 23F and ST433-serotype 22 clones
– Fam1/clade2 (n = 3) was associated with ST2217-serotype 35F clone
– Fam2/clade3 (n = 39) was associated with Spain23F-1, Spain9V-3, Netherlands3–31, Netherlands7F-39, ST110-serotype 18C, ST62-serotype 11A, ST202-serotype 19A, ST247-serotype 4, ST30-serotype 16, ST67-serotype 9N and ST88-serotype 19F clones
– Fam2/clade4 (n = 3) was associated with Sweden15A-25, Netherlands18C-36, ST42-serotype 23A, ST2312-serotype 8 and ST558-serotype 35B clones
– Fam2/clade5 (n = 3) was associated with Poland6B-20, ST260-serotype 3 and ST989-serotype 12 clones.
Conclusions: A clear association between PspA (Fam/clade) and genotype (ST) was found, whereas PspA (Fam/clade) was independent of serotype. The most important Fam/clades of PspA among invasive pneumococci isolated from adult patients in our geographical area were Fam2/clade3 and Fam1/clade1. Since international clones contain PspA Fam1 and Fam2, both families should be included in a future PspA based vaccine.

P1332 Comparative analysis of Bordetella pertussis strains included in the Serbian whole-cell vaccine

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Objectives: In Europe, countries use different vaccination strategies against pertussis. In Serbia, vaccination against pertussis has been in use for 50 years. The whole-cell pertussis vaccine has been manufactured in the Torlak Institute, Belgrade, Serbia since 1957.
The vaccine is made of autochthonous Bordetella pertussis strains, and "new" strains have been added to the vaccine for decades. Current composition of the vaccine has been used since 1985, and contains four strains: 1772/57 (year of isolation, 1957), 2047/57 (1957), 23/81 (1981) and 8/84 (1984). Because antigenic divergence has been found between B. pertussis vaccine strains and clinical isolates in many European countries, we wanted to analyse and compare these vaccine strains.
Methods: The analysis included serotyping of fimbriae (Fim) by slide agglutination, genotyping of pertussis toxin S1 subunit (ptxA) and pertactin (prn) by sequencing and LightCycler PCR, and DNA fingerprinting by pulsed-field gel electrophoresis (PFGE). The band patterns obtained by PFGE were analysed using the computer software BioNumerics. Different profiles were defined by band differences in the DNA band patterns and compared with those from international reference strains, Finnish vaccine strains (1772 and 18530) and clinical isolates.
Results: All three serotypes Fim2 (8/84), Fim3 (23/81) and Fim2,3 (1772/57 and 2047/57) were confirmed among the four vaccine strains. The strains 1772/57 and 2047/57 represented ptxA2, and strains 23/81 and 8/84 were ptxA1.The prn2 occurred only in strain 8/84, and the other three demonstrated prn1. Four different PFGE profiles were found: BpSR23 (1772/57), BpFINR1 (2047/57), BpFINR9 (23/81) and BpSR46 (8/84). Strain 1772/57 had the same PFGE profile as the Finnish vaccine strain 1772. Strain 2047/57 had the profile predominant among the Finnish strains isolated in the 1950s-1960s. The profile of 23/81 was prevalent in Finland in the 1970s, and the profile of strain 8/84 was found among the Finnish isolates from the 1970s and early 1990s. The Finnish vaccine strains represented ptxA2 or ptxA3/prn1, whereas the current circulating strains represented ptxA1/prn2.
Conclusions: The Serbian vaccine strains showed differences in ptxA and prn as well as in the PFGE profiles. The PFGE profiles of the vaccine strains were similar to the Finnish isolates from the same decades and different from the current Finnish circulating strains.

P1333 Evaluation of a new meningitis vaccine in healthy African population: hope on the horizon

B.J. Okoko on behalf of the MVP Trial Group

Introduction: Recurrent severe epidemics of meningococcal disease strike the meningitis belt extending from Senegal to Ethiopia. Annual incidences can reach 1,000/100,000 vs. 1/100,000 in developed countries. Group A meningococcus has remained unique in its ability to cause large epidemics. A vaccine that induces long-lasting protection and herd immunity is urgently needed. The Meningitis Vaccine Project (MVP) was funded in 2001 as a partnership between WHO and PATH to accelerate the development and introduction of affordable meningococcal conjugate vaccines. MVP's goal is to eliminate meningococcal epidemics in sub-Saharan Africa. A new MenA conjugate vaccine, manufactured by Serum Institute of India Ltd Pune – India, PsA-TT was safe and immunogenic with durable immunity when tested in a Phase I study in Indian adults. The vaccine is currently being tested in two phase II/III clinical studies where safety and immunogenicity are being studied among African population 1–29 years of age.
Method: Two observer-blind, randomised, controlled studies to assess safety, immunogenicity, induction of immune memory, antibody persistence of PsA-TT and pharyngeal carriage of Meningococcus in African toddlers aged 1–29 years are underway in Gambia, Mali and Senegal. After parental informed consent, each participant received a single intramuscular injection of study vaccine (PsA-TT), reference vaccine (ACYW135), and control vaccine (*Haemophilus influenzae* b) in the MVP 002 trial. Blood samples were obtained prior to immunisation and 4 weeks later for serum bactericidal antibody (SBA) activity and anti-polysaccharide group A (anti-PsA) IgG levels. Participants were followed for safety evaluation for 4 weeks.
Results: Preliminary result is available for the 601 toddlers aged 12–23 months from Gambia and Mali. PsA-TT was safe; rSBA GMT at baseline were 14, 16 and 13 for PsA-TT, PsACWY and Hib-TT; and rose to 6234, 365 and 61 with four-fold rises observed in 96%, 64% and 36% of recipients respectively, at week 4. 900 children and young adults aged 2–29 years have been enrolled in Gambia, Mali and Senegal for safety evaluation, antibody persistence and pharyngeal carriage. There is no safety issue to date and the study is on-going. The MVP 002 booster results soon to be available will be presented.
Conclusion: These unambiguous data are encouraging as MVP and partners continue their efforts to develop an affordable Men A conjugate vaccine for Sub-Saharan Africa.

P1334 The serotype-specific efficacy and immunogenicity of a 9-valent pneumococcal conjugate vaccine determined during an efficacy trial in The Gambia, West Africa

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Background: A large randomised, double-blind, placebo-controlled vaccine trial in The Gambia showed that a 9-valent pneumococcal conjugate vaccine (PCV-9) had high efficacy against radiological pneumonia and invasive pneumococcal disease of vaccine serotype and reduced substantially hospital admissions and child mortality. Here, we report post vaccination pneumococcal antibody concentration in a nested subsample of infants who participated in this trial.

Methods: A single 2–4 ml venous blood specimen was collected from 213 4–6 weeks Gambian children after the third dose of vaccine or placebo and tested by ELISA for type-specific IgG antibodies to pneumococcal serotype 1, 4, 5, 6B 9V 14 18C, 19F and 23F polysaccharides. And from the main trial, we performed per-protocol and intention-to-treat analyses to assess the serotype specific vaccine efficacy.

Results: Geometric Mean Concentrations (GMCs) were significantly higher for each serotype in children receiving three doses of PCV-9 than those in placebo group. Among PCV-9 recipients, GMCs ranged between 2.61 and 11.09 μg/ml with the highest being against serotype 14 and the lowest against 9V. Point estimates of the proportion of infants with post vaccination antibody concentrations $\geqslant$0.15 μg/ml, $\geqslant$0.35 or $\geqslant$1.0 μg/ml ranged from 97.9% – 100%, 92.9% – 100% and 83.9% – 95.8%, respectively, for individual polysaccharides. In contrast, only 9.6% – 39.0%, 7.2% – 20.9% and 1.1% – 10.1% of infants in the placebo group attained these antibody concentrations. The estimated overall protective level for all the 9 serotypes, based on the vaccine efficacy against vaccine type specific invasive pneumococcal disease of 77% (95% CI: 51, 90) observed in the whole trial, was 2.3 μg/ml(95% CI: 1.0, 5.0).

Conclusion: The 9-valent pneumococcal conjugate vaccine studied was immunogenic in a Gambian population where it was also found to be efficacious

P1335 Serum bactericidal antibody response to polysaccharide meningococcal vaccination in common variable immunodeficiency and its clinical implications

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Objectives: Common variable immunodeficiency (CVID) is a heterogeneous group of disorders, characterised by hypogammaglobulinaemia and recurrent bacterial infections. Some patients with CVID make a successful immune response to polysaccharide vaccine. In this study, we examined the clinical features of those patients with CVID who respond poorly to polysaccharide meningococcal vaccine.

Methods: Twenty-five cases with CVID (18 male and 7 female) and 25 healthy volunteers received meningococcal polysaccharide vaccine A + C. Serum bactericidal antibody (SBA) titres were measured at baseline and after 3 weeks. Response was correlated with clinical and immunological manifestations of CVID.

Results: The serum bactericidal geometric mean titres (GMT) post-vaccination in the CVID patient group was 7.36, which was much lower than the control group (12.13). Twenty-four of 25 controls (96%) had a protective SBA titre of $\geqslant$8 post vaccination, whereas only 16 of 25 CVID patients (64%) had a protective titre (P-value=0.013). Amongst the cases of CVID who were non-responders, there was a significantly increased rate of bronchiectasis (P =0.008), splenomegaly (P =0.016), and autoimmunity (P =0.034) in comparison with patients who had protective SBA titres. A reversed CD4/CD8 ratio was more common in the non-responder group of patients.

Conclusion: The responder patients may have a good prognosis, while non-responder patients may have undefined immune abnormalities leading to several complications, such as bronchiectasis, opportunistic infections, splenomegaly, and autoimmunity. Therefore, vaccination response may provide a novel functional sub-classification of CVID patients, enabling accurate prognostic evaluation to identify high-risk patients at the time of diagnosis.

P1336 Changes in the epidemiology of invasive pneumococcal disease in adults after introduction of PCV7 in children, Barcelona, Spain

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Objectives: The PCV7 pneumococcal vaccine for children was licensed in June 2001 in Spain. Currently, the estimated uptake of PCV7 is about 40–50%. The aims of this study were to analyse the distributions of serotypes and genotypes and the changes in the incidence of invasive pneumococcal disease (IPD) in adult patients in our geographical area in the pre- and post-vaccine periods.

Methods: A total of 1200 cases of IPD collected in 3 hospitals in our area in Barcelona were enrolled in order to estimate the incidence of IPD. Only pneumococci isolated from adult patients admitted to Hospital Universitari de Bellvitge (n= 876) were available for serotyping and molecular typing (PFGE and MLST). Antibiotic susceptibility was performed by microdilution.

Results: The most frequent serotypes throughout 1997–2006 period were 3 (11.3%), 14 (8.3%), 1 (7.8%), 9V (6.2%), 19F (4.9%), 4 (4.8%), 5 (4.4%), 19A (4.4%), and 23F (4.0%), and the dominant genotypes were Spain9V-3 (11.4%), ST260-serotype 3 (6.7%), Sweden1–28 (5.6%), and Spain23F-1 (4.5%). When 1997–2001 and 2002–2006 periods were compared, the following changes were observed (all $p < 0.05$): a) An increase in the incidence of IPD (cases/100.000 persons) from 12.9 to 16.4 for all adults and from 7.9 to 10.6 for adults 15–64 years old; b) The incidence of IPD caused by non-vaccine serotypes rose from 7.8 to 11.6 for all adults, from 5.0 to 7.5 for persons 15–64 years old and from 22.9 to 32.0 for persons >64 years old; c) The incidence of IPD caused by vaccine serotypes fell 22% (95% CI, −41 to 3) for persons >64 years old; d) A decrease of vaccine-related clones (Spain23F-1, Spain6B-2, Spain14–5, ST247-serotype 4 and ST88-serotype 19F); e) An increase of non-vaccine serotypes 7F, 24 and 35B and non-vaccine clones (Sweden1–28, Denmark14–32, Netherlands7F-39, ST989-serotype 12, ST433-serotype 22 and ST558-serotype 35B); f) A decrease of penicillin-, tetracycline-, chloramphenicol- and cotrimoxazol-resistance.

Conclusion: An increase of incidence of IPD was observed in adult patients in our area in 2002–2006 period, and it was associated with an increase of the incidence of IPD caused by non-vaccine serotypes. The incidence of IPD caused by vaccine-serotypes remained stable among persons 15 to 64 years of age, whereas it felt for adults over 64. A significant decrease in antibiotic resistance rates related to decrease of multiresistant clones was observed in our geographical area.

P1337 The evaluation of three pro-inflammatory cytokines

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Objective: Smooth Lipopolysaccharide (S-LPS) is the most antigenic structure expressed on the surface of *Brucella* smooth strains which promotes the innate and adaptive immune responses. LPS-induced innate immune responses are involved in the proinflammatory reactions and the release of proinflammatory cytokines, such as TNF-α, IL-1Beta and IL-6. The controlled increase of proinflammatory responses would be achieved by the application of adjuvants and studied as a method to induce the innate immune responses against the LPS of *Brucella*. Outer membrane vesicle of *Neisseria meningitidis* is one of the newly studied adjuvants with microbial origin and Several studies

carried-out about the probability of *Neisseria meningitidis* OMV application as an adjuvant. We have analysed the induction of three proinflammatory cytokines(TNF-α, IL-1β and IL-6)in immunised animal models with extracted S-LPS of *B. abortus* S99 complexed with OMV of N.meningitids, as an adjuvant.
Methods: The S-LPS of *Brucella* was extracted by hot phenol-water method. Then extracted S-LPS injected to mice in two different patterns, 1: *B. abortus* S-LPS 2: *B. abortus* S-LPS-*N. meningitidis* OMV (OMV as a probable potent adjuvant) noncovalent complex. After injections, animals were bled in 7, 14, 21 and 28'th and Finally sera analysed to detect the level of TNF-alfa, IL-1β and IL-6 by ELISA method.
Results: IL-6 was the first detected cytokine one week after the first injection. Peak amount of IL-6 detected two weeks after the first injection and there was not a significant difference between IL-6 amounts in LPS and LPS-OMV immunised groups. TNF-α and IL-1β were not detected in serum of S-LPS-immunised animals after the injections but interestingly both of these two cytokines were detected in the serum of animal inoculated with the S-LPS-OMV complex. The peak amounts of IL-1β and TNF-α were detected three weeks after the injection.
Conclusion: OMV can efficiently induce the production of IL-1β and TNF-α in the studied animal models. Since IL-1β and TNF-alfa are cytokines with innate cellular immunity sources and innate immunity is an important primary barrier against *Brucella* infection, we can conclude that OMV could be applied as an effective adjuvant in the candidate vaccines to elicit a more potent cell mediated innate immune response, in parallel with the augment of specific humoral and cellular responses against *Brucella* LPS.

P1338 Evaluation of pro/anti inflammatory responses after rectal administration of *Mycobacterium bovis* BCG

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Objectives: Rectal administration of BCG induces a protective immune response against tuberculosis in animal models interestingly without any inflammatory complications. To understand the lack of inflammation we evaluated expression pattern of pro/anti inflammatory agents in the colorectal epithelial cell lines and fresh prepared epithelial cells (ECs) in presence or absence of intraepithelial lymphocytes (IELs). The regulatory mechanism of these expression profiles was studied by comparison of NFkB and PPARg activity.
Methods: IL-8 secretion was measured in Caco-2, HT-29 and SW-480 cell lines challenged with BCG by ELISA method. NFkB activity of the cell lines was assayed by DNA-based ELISA. IL-8 and IL-4 secretion was measured in guinea pig and macaque after rectal immunisation of BCG and also in their corresponding ECs and ECs+IELs. PPARg activity of macaque EC and ECs+IELs was measured by DNA-based ELISA. mRNA expression profile of some cytokines and chemokines in mice vaccinated with rectal BCG was determined using RNase protection assay (RPA).
Results: In response to BCG, IL-8 increased in a time dependent manner. This increase was corresponded by increase in NFkB activity. Guinea pig and macaque ECs showed the same behaviour by remarkable increases in IL-8 level. However ECs+IELs challenged with BCG didn't increase IL-8. We demonstrated a dramatic increase of 4 and 7 fold in IL-4 levels in ECs+IELs. Also PPARg activity was doubled during 24 hours in ECs. Two and seven days after BCG administration in mice, RPA was run on RNA from total colon, ECs and EC+IELs. The expression of pro-inflammatory cytokines IL-6, IL-18, IL-12p40, IL-1b, IFNg and MIF was increased in ECs lonely, whereas this effect was followed only by 2 days in ECs+IELs or in total colon. But in 7 days after BCG stimulation, the levels of these inflammatory agents were normalised. The same pattern was observed for the chemokines MIP-2 and MCP-1 while RANTES and IP-10 increased remarkably upto day seven. Anti-inflammatory cytokine IL-10, undetectable in ECs, was highly increased in ECs+IELs and also in total colon.
Conclusion: Rectal delivery of BCG induces an early balanced inflammatory process orchestrated by pro-inflammatory agents and Th2 mediated modulations that are controlled by a late IL-10 response. This balance is mainly due to IELs interactions which stable homeostasis at the colorectal mucosal surfaces.

P1339 Recombinant bacterial ghosts as a new vaccination approach against *Helicobacter pylori* infection

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Objectives: Genetic inactivation of Gram-negative pathogenic bacteria through controlled expression of cloned lysis gene E originated from bacteriophage PhiX174 has offered new insights in developing non-living vaccines specially those meant for oral administeration. Bacterial ghosts are empty cell envelopes derived mostly from Gram-negative bacteria by controlled expression of cloned lysis gene E. The induced trans-membrane tunnels in the cell wall expels the cytoplasmic contents while maintaining the antigenic properties of a living intact cell.
The high prevalence of *Helicobacter pylori* (Hp) infection is a major health problem worldwide especially in developing countries such as Iran, thus demanding the development of efficient vaccine strategies to prevent infection in early childhood. Since bacterial ghosts can mimic bacteria in structural properties, Hp ghosts expressing immunogenic proteins maybe a suitable approach.
Methods: omp18 gene was amplified from a local Hp strain through gene-specific PCR with designed primers according to published sequences in the GenBank and cloned under a chemical inducible promoter into Hp/*E. coli* shuttle vector.
The second antibiotic selection marker was also cloned in the above recombinant vector. Protein expression was induced in *E. coli* by IPTG and confirmed via immuno blotting using true Hp positive and negative sera. *E. coli* cells were co-transformed with lysis and expression vectors. Developing bacterial ghosts was performed by elevating the temperature to 42C after primary IPTG induction of Omp18 expression.
Results: The nucleotide homogeneity of Iranian omp18 gene was up to 95%. Kanamycin resistance gene (Kanar) was cloned in the recombinant vector as the 2nd antibiotic marker. The specific 25kDa Omp18 protein was observed on 15% SDS-PAGE and its immunogenicity was confirmed via immunoblotting. Co-expression of omp18 and lysis genes was performed and generation of bacterial ghosts expressing Hp Omp18 was confirmed by blotting.
Conclusion: The expression of recombinant Omp18 protein and bacterial ghost production in *E. coli* system has been confirmed. The expression of two cloned genes (Omp18 and Lysis E protein) is being optimised and the constructed ghosts are going to be examined in a helicobacter animal model employing C57BL/6 mice.

P1340 Sources of infection of pertussis in infants too young to be vaccinated

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Objectives: Despite high vaccine coverage (96%), pertussis is endemic in The Netherlands.
Several changes in the vaccination schedule have been implemented: introduction of an acellular preschool booster in 2001 and replacement of a whole-cell vaccine by an acellular vaccine since 2005. Still, infants too young to be vaccinated are at greatest risk for severe pertussis. To investigate the optimal vaccine strategy to protect infants, we determined their source of infection.
Methods: From February 2006 until December 2008, all paediatricians, microbiologists and public health personnel are asked to notify any hospitalised child younger than 6 months of age with pertussis. From household members of the hospitalised infant, a nasopharyngeal swab and buccal cells will be obtained for PCR or culture to identify B. pertussis or B. parapertussis. Furthermore, IgG-PT and IgA titres are assessed and clinical symptoms and vaccination history are determined.

Results: In the first 18 months of study, 109 index patients and 411 household members were enrolled. Of the latter, 200 (49%) had laboratory confirmed B. pertussis infection and 4 (1%) B. parapertussis infection. Of the infected household members, 88% were vaccinated. For 73% of these typical cough symptoms were reported, with a median duration of 30 days. Three children and one adult were hospitalised. Preliminary results indicate that 25% of the mothers, 10% of the fathers and 21% of the siblings in the study had introduced pertussis in the household and thus most likely transmitted the infection to the infant.
Conclusions: Preliminary results demonstrate that siblings and mothers are most often the source of infection of pertussis in infancy in the Netherlands. Vaccination of household members of infants may eliminate a substantial part of pertussis disease burden in infants. Besides, symptomatic pertussis infection occurs frequently in adults. Dynamic modelling will be used to determine what vaccination strategy is the most (cost-)effective way to reduce disease burden due to pertussis, with prevention of pertussis among infants as main focus.

Diagnosis, pathogenesis and in vitro studies in fungal infections

P1341 Diagnosing fungaemia faster

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Objective: Rapid initiation of appropriate antifungal treatment is associated with a reduction in mortality. Unfortunately species identification and susceptibility testing is time consuming. Recently, a commercial susceptibility testing card to the VITEK system was developed, and we decided to evaluate this in a routine laboratory in comparison with the EUCAST reference method.
Methods: A strain collection of 20 yeast isolates from blood cultures (8 *C. albicans*, 6 *C. glabrata*, 2 *C. tropicalis*, 2 *C. parapsilosis*, 1 *C. krusei* and 1 *Cryptococcus neoformans*), were tested by VITEK® by the AST-YS01 card and the results were correlated to the MICs obtained by the EUCAST method performed at the reference laboratory. The VITEK resistance card includes amphotericin B, fluconazole, voriconazole and flucytosine. The EUCAST testing was performed as part of a surveillance scheme and included amphotericin B, fluconazole, voriconazole, itraconazole, posaconazole and caspofungin. Agreement was defined as identical classification in S, I/SDD/ and R.
Results: There were 100% agreement for amphotericin B susceptibilities, and all the strain's MICs were $\leqslant 1$ μg/mL.
For 17:20 isolates the interpretations were in agreement for the azoles if S $\leqslant 2$ μg/mL for fluconazole, S $\leqslant 1$ μg/mL for voriconazole. Two strains showed minor disagreements for fluconazole, with MICs of 0.5 and 2 μg/mL by the EUCAST, and both 4 μg/mL by the VITEK method. One *C. tropicalis* showed high level resistance towards fluconazole and voriconazole by the EUCAST method, MICs >16 and >4 μg/mL, but by VITEK the MIC's were <1 and <0.12 μg/mL, respectively. This strain will be further examined. Flucytosine susceptibilities were tested by VITEK, and only the *C. krusei* were found resistant, MIC=16 μg/mL. The VITEK results were ready after median 18 h (range 13–21).
Conclusion: The susceptibility profiles for the antifungals included in the card were reliably done overnight from colonies on agar plates. Including an resistance card when using identifications cards by VITEK, may be less time consuming, and is economical attractive compared to our routine methods. There is two disadvantages so far, no echinocandins are yet included in the card, and there is not yet any easy procedure for using samples directly from the blood culture bottles.

P1342 Evaluation of two chromogenic *Candida* agars: need for additional training to optimise results

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Objectives: To evaluate two chromogenic agars for the identification of clinically relevant yeasts: OCCA (Oxoid) and Candiselect4 (Biorad). Candiselect4 allows species identification of *Candida albicans*, *Candida glabrata*, *Candida krusei* and *Candida tropicalis*. OCCA agar identifies the same species, as well as *Candida dubliniensis*, *Candida parapsilosis*, *Candida kefyr* and *Candida lusitaniae*, but recommends to classify isolates into 4 clinical relevant groups (*C. glabrata* + *C. parapsilosis*; *C. albicans* + C.dubliensis; *C. krusei*; *C. tropicalis*). The second objective was to evaluate if the % correct identification (PCI) could be improved by additional training of laboratory technicians.
Methods: 104 well characterised clinical isolates (from fungaemias) were included. Species distribution was: *C. albicans* (52), *C. glabrata* (33), *C. krusei* (6), *C. parapsilosis* (6), *C. tropicalis* (4), *C. inconspicua* (1), *C. famata* (1) and *Cryptococcus neoformans* (1). After incubation (48 h, 37°C) 14 lab technicians identified each culture. Educational material was used concomitantly. The evaluation was repeated after additional training of lab technicians.
Results: Before additional training, PCI was poor with OCCA for identifications at the species level. Misidentification, even between *C. albicans* and non *C. albicans* species, was frequent. After the classification into the 4 clinical relevant groups, PCI increased. Both PCI per species and per group improved significantly after training: a single *C. albicans* isolate was misidentified by 1 person only. For Candiselect4, differences in PCI before and after training were less clear (Table).

Species	PCI					
	on OCCA				on Candiselect4	
	Species identification		Group classification		Species identification	
	Before training	After training	Before training	After training	Before training	After training
C. albicans	82	92	95	99.8	99	100
C. glabrata	57	71	92	99	71	72
C. krusei	97	100	97	100	100	100
C. tropicalis	71	72	71	72	56	64
C. parapsilosis	53	28	see *C. glabrata*		Not applicable	

Conclusion: To reach a correct identification of *Candida* isolates on chromogenic agars, additional training is required. After this training, PCI increased for species identification as well as for classification into the 4 clinical relevant groups, with a higher improvement for OCCA than for Candiselect4. PCI for the *C. glabrata* + *C. parapsilosis* group and *C. tropicalis* was significantly better on OCCA compared to Candiselect4.

P1343 Assessing the role of a fungal blood culture vial in the diagnosis of *Candida* spp. bloodstream infection in an Irish tertiary referral centre

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Candida blood stream infection is an important cause of morbidity and mortality in hospitalised patients. Diagnosis is primarily based on isolating *Candida* spp. in blood cultures, although these have been found to be negative in approximately 50% of histologically proven invasive candidiasis at autopsy. This hospital uses conventional aerobic (BD Bactec plus Aerobic/F TM) and anaerobic (BD Bactec Plus AnaerobicTM) vials as well as a selective fungal culture vial (BD Bactec Mycosis-IC/F) in an attempt to optimise the diagnosis of candidaemia in high risk patients.
Objective: The aim of this study was to quantify the diagnostic benefit of including a selective fungal blood culture vial with the conventional set.
Methods: All blood cultures yielding *Candida* spp. in 2005 and 2006 were retrospectively reviewed. Where conventional aerobic and anaerobic vials, as well as a selective fungal vial were collected, we analysed the

paired sets to determine whether the fungal vial increased the sensitivity or reduced the time to positivity in recovering *Candida* spp.

Results: In the period studied, there was a total of 167 blood culture isolates of *Candida* spp. from 67 patients. There were 52 sets where paired conventional and fungal blood culture vials were received. Of these, both sets were positive in 24 cases (46%), the conventional vials only were positive in 14 (27%) and the fungal vial only was positive in 14 (27%), which gave an additional yield of 36%. In addition, there was a trend towards more rapid detection of *Candida* spp. from the fungal vials with 69% positive within 48 hours, compared to 48% in the case of the conventional sets.

Conclusion: The inclusion of a selective fungal vial with conventional aerobic and anaerobic vials improves the recovery rate of *Candida* spp. from blood by 36%. The time to recovery of *Candida* spp. is also reduced, with an additional 21% detected within 48 hours. This is clinically important in a disease where time to treatment improves survival.

P1344 The culture and antifungal sensitivity testing of pathogenic fungi on a highly porous ceramic

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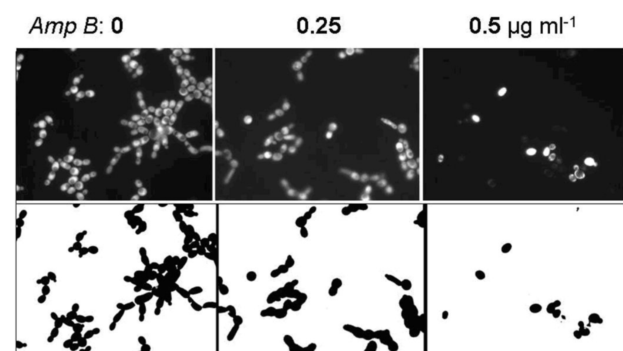
Objective: To develop a fast and widely applicable, culture-based antifungal sensitivity testing (AST) assay developed around the growth and in situ imaging of fungi on a porous ceramic support.

Methods: Sterile highly porous ceramic (PAO) strips (Anopore, an inert nanoporous ceramic derived from aluminium oxide) were placed upon agar plates (Sabauroud media or RPMI) containing defined concentrations of antifungals. The strips were then inoculated with the fungi (yeast, *Trichophyton*) to be tested, incubated, stained with fluorogenic dyes and imaged by fluorescence microscopy. Digital mages were processed in ImageJ software [1] to determine the effect of the antifungal agent.

Figure: Examples from AST of *Candida albicans*. Upper row shows a raw image of the yeast cultured on PAO showing growth inhibition with increasing concentrations of amphotericin B (AmpB). Lower section shows processed image used to calculate microcolony areas.

Results: A ssignificant effect of ampB on *Candida* was demonstrated within 90 min. MIC values could be derived reliably with 4 h culture (amphotericin B and caspofungin) or 7 h (voriconazole, itraconazole). For a total of 74 assays, in 65 cases the assignment by PAO (sensitive [S], resistant [R], intermediate [I]) was identical to the E-test. Where differences occurred these were always single category disagreements. Testing of *Trichophyton rubrum* and other filamentous fungi was possible with 16 h culture time.



Conclusions: Microcolony imaging is a rapid (c. 5× faster than E-test) and accurate approach to AST testing. Fungi grow well on PAO, adhere to the material and the images are easy to process. Performing these tests on PAO facilitates staining and imaging in situ and has allowed rapid assessment of whether an antifungal was inhibitory to growth. The method could be improved by multiplexing (more than one antibiotic/strip), reinforcing the PAO to facilitate handling and using cheaper dyes. Given that the method is also applicable to bacteria [1] including TB [2] PAO may be generally usable for antimicrobial testing and for applications outside clinical microbiology [3]. The PAO approach may also be useful for the study of heterogeneity of response to antimicrobials and other research applications.

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P1345 Clinical and management utility of *Candida albicans* germ tube antibody detection in critically ill patients. Influence on mortality

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Background: Invasive candidiasis in critically ill patients represents a diagnostic challenge. Recently, an immunofluorescence test for *Candida* germ tube antibody (CAGTA) detection has been marketed. The aims of the present study were to determine the prevalence of CAGTA in an ICU setting, the clinical and management utility of its detection, and the influence of a strategy based on early determination of CAGTA on mortality in a selected ICU population.

Methods: A prospective and observational multicentre study was developed in six Spanish University hospitals during 2005. CAGTA test was performed twice a week if predetermined risk factors were present, and a positive result was considered if the testing serum dilution was equal or higher than 1:160 in at least one sample. Clinical, microbiological and outcome variables were recorded. A multivariate analysis was performed to assess the influence of this technique on mortality.

Results: Fifty-three critically ill patients non-neutropenic (37.7% post surgery) were included. Twenty-two patients (41.5%) had CAGTA-positive results, none of them with positive blood culture for *Candida*. The intra ICU mortality rate was significantly lower ($p = 0.004$) in positive CAGTA patients (61.2% vs. 22.7%). A multivariate analysis confirmed a CAGTA-positive result as the only protective factor independently associated with ICU mortality (beta coefficient = −0.3856; 95%IC: −0.648; −0.123).

Conclusions: The rate of CAGTA-positive results in a selected group of ICU patients is high. A strategy based on early determination of CAGTA might reduce the ICU mortality of patients with risk factors to develop invasive candidiasis.

P1346 Serum procalcitonin measurement contribution to the early diagnosis of invasive candidiasis in critically ill patients: findings from the CAVA study

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Background: Invasive candidiasis (IC) are life-threatening infections in the intensive care units (ICUs). Outcome could be improved by the early administration of antifungals. Risk factors assessment by scoring systems such as the "Candida Score" (CS) including the search for multifocal fungal colonisation (MF) has been recently proposed for the selection of the patients who will actually develop IC among the colonised ones. Procalcitonin (PCT) is useful for the early diagnosis of sepsis but its behaviour is unclear in the setting of IC. We investigated PCT variations in the patients with MF with or without IC.

Methods: A prospective observational study (The Candida Score Validation Study) including every nonneutropenic critically ill patients hospitalised for more than 7 days was conducted in 35 ICUs in Spain, Portugal and France between April 2006 and June 2007. Risk factors for IC used for the calculation of the CS (i.e., fungal colonisation, surgery,

total parenteral nutrition and severe sepsis) were assessed, and serum were collected at the time of inclusion and weekly thereafter. The patients with MF but without concurrent bacterial infection were randomly selected for the measurement of PCT (Kryptor, Brahms Diagnostic). Risk factors for IC were analysed. ROC curves were constructed for predictive value assessment of PCT and CS.
Results: 147 PCT dosages were performed in 63 patients (MF: n = 33; IC: n = 30). Patients from each group were comparable regarding age, gender and APACHE II. However, the patients with IC were more likely to be surgical ($p < 0.001$) and to have a high SOFA score ($p = 0.008$). PCT after a 7-day hospitalisation was found to be significantly more elevated in the MF+IC group (1.05±1.93 ng/mL), than in the MF (0.63±2.00 ng/mL) ($p = 0.007$), while next values were comparable. The highest Candida Score value (CSmax) calculated throughout the patient stay was the sole independent predictor of IC. The ROC curves analysis showed that the predictive values of PCT, and CSmax were comparable (AUC=0.713, and 0.727, respectively; $p = 0.863$).
Conclusion: PCT after a 7-day hospitalisation is more elevated in the patients with MF who will develop IC thereafter than in those who will not, despite its elevation remains mild. The higher proportion of surgical patients and the higher degree of organ dysfunction could account for such variations. PCT could however be considered for the IC risk assessment in addition to the Candida Score.

P1347 Fungal colonisation of gastro-intestinal tract, anti-*Saccharomyces cerevisiae* antibodies and anti-mannan *Candida* antibodies in patients with inflammatory bowel disease and irritable bowel syndrome

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Introduction: A search for serologic markers useful in diagnostics, including differential diagnostics of inflammatory bowel disease (IBD) continues. The idea that microorganisms play a role in the aetiology of IBD has gained ground considerably in recent years. Antibodies against mannans of *Saccharomyces cerevisiae* (ASCA) were observed in Crohn's disease in 1988. It is said that anti-mannan *Candida* spp. antibodies (AMCA) may be useful in diagnostics of often latent fungal infections. Therapy with antibiotics as well as immunosuppressive treatment favour fungal infections especially in the presence of intestinal mucosa lesions. The aim: to assess the level of ASCA and AMCA and the incidence and intensity of fungal colonisation of gastrointestinal tract in IBD patients in comparison with irritable bowel syndrom (IBS) patients.
Materials and Methods: 42 patients suffering from ulcerative colitis (UC) and 26 patients with Crohn's disease (CD) as well as 19 IBS patients were subjected to the study. The level of antibodies was assessed with ELISA. The quantitive and qualitative fungal cultures of the stool were performed in all the subjects using Sabouraud agar plates supplemented with chloramphenicol and Chromagar-*Candida* ID-32-Biomerieux test.
Results: The results were obtained as shown in the table.

Study group	ASCA	AMCA	SFC
CD (n = 26)	14/24 (58.3%), SEM: 77.4±75.6	6/26 (23.1%) SEM: 7.8±5.0	1/26 (3.9%)
UC (n = 42)	1/15 (6.7%) SEM: 12.9±14.5	6/42 (14.3%) SEM: 10.7±5.6	3/42 (7.1%)
IBS (n = 19)	5/15 (33.3%) SEM: 16.7±11.6	7/19 (36.8%) SEM: 10.1±7.0	5/19 (26.3%)

n = number of subjects; SEM = mean value, SFC = significant fungal colonisation ($>10^5$ CFU per 1 g of stool).

Conclusions:
1. ASCA were found in 58.3% of CD, 33.3% of IBS and 6.7% of UC patients.
2. The level of ASCA in CD patients was significantly higher then in UC and IBS patients ($p = 0.002$ and $p = 0.003$, respectively).
3. The increased level of AMCA and significant fungal colonisation were observed more often in IBS patients then in IBD patients.

P1348 Confirmation of positive galactomannan results in serum by detection of 1,3-beta-D-glucan could eliminate false positive results of the test

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Objectives: Detection of galactomannan (GM) by Platelia *Aspergillus* assay is widely used for early diagnosis of invasive aspergillosis (IA). One of the main disadvantages of this test are false positive results that might be caused by many factors (administration of antibiotics, gluconate-containing solutions etc.). To be able to recognise false positive results in short time is of a great medical interest. One of the possibilities is to confirm the detection of GM by detection of other "panfungal" antigen – 1,3-beta-D-glucan (BG) – in the sample.
Methods: From 2/2007 to 10/2007 4012 samples from 320 patients were tested for GM using Platelia *Aspergillus* kit. Patients with IP > 0.5 in 2 consecutive samples have been marked as GM positive. These 2 GM positive serum samples have been subsequently tested for BG by Fungitell test. Samples with results >60 pg/ml were marked as BG positive. All GM positive patients were classified according to EORTC/MSG criteria (2002) for probability of IA.

	GM in 2 samples	GM in 2 samples + BG in 1 sample	GM in 2 samples + BG in 2 samples
Total positive pts.	39	20	9
No. of pts. with IA	6	6	6
PPV for diagnosis of IA	**16%**	**30%**	**66%**
Sensitivity for diagnosis of IA	100%	100%	100%

Results: From 320 patients tested 39 patients had two consecutive samples GM positive (were marked as GM positive pts.). However, proven and probable IA was found only in 6 of them (PPV = 15%). Thus in the majority of pts (33 = 85%) the result of the Platelia *Aspergillus* assay was false positive.
Fungitell gave positive result in 27 from 78 GM positive samples (35%). 20 pts. (51%) with consecutive GM positivity had also at least one of the two samples positive for BG, giving PPV 30%. Only 9 pts. (23%) had the 2 consecutive samples both GM and BG positive. PPV of the GM test thus increased to 66% when GM positivity was confirmed by BG positivity in two consecutive samples. Sensitivity for diagnosis of IA remained 100%.
Conclusion: Confirmation of GM positive results by testing for BG positivity increased the PPV of the Platelia *Aspergillus* test from 15% to 66%. This approach did not decrease the sensitivity of the GM assay and using already obtained samples did not lead to the delay in IA diagnosis. Using the combination of the results of these two tests might help to identify false positive GM results and subsequently reduce the possibility of over treatment.
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P1349 3rd Nordic distribution of external quality assessment samples in medical mycology: do we still need to improve?

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Objectives: A Nordic External Quality Assessment program in medical mycology was established in 2005. In order to monitor the level of routine diagnostics, not "best practice", the specimens were designed to resemble clinical samples and the laboratories were asked to handle the samples like routine samples.

Methods: Five simulated clinical samples were distributed at no cost to 63 Nordic laboratories of clinical microbiology of whom 54 submitted results. The specimens contained the following microorganisms: (1) *Fusarium solani* (corneal scraping), (2) *Candida* (*C.*) *albicans* and *C. krusei* in a ratio of 10:1 (blood culture), (3) *C. glabrata* and *Stenotrophomonas maltophilia* (tracheal aspirate), (4) *Aspergillus* (*A.*) *fumigatus* and *C. dubliniensis* (BAL), and (5) *C. inconspicua* (vaginal secretion). A brief clinical information was given for each specimen.
Results: Specimen 1: 48% (26/54) of the laboratories detected the mould, of whom 6 correctly identified it to the species level (23%). Specimen 2: 83% (45/54) reported the presence of the *C. krusei* isolate, but only 57% (31/54) also reported the *C. albicans* isolate even though the ratio was in favour of *C. albicans*. Specimen 3: 94% (51/54) detected the yeast, of whom 86% (44/51) correctly identified it as *C. glabrata*. Specimen 4: The presence of *A. fumigatus* was correctly reported by 59% (32/54) of the laboratories while 6% (three laboratories) did not report growth of a mould at all. Specimen 5: 17% (9/54) correctly reported the isolate as *C. inconspicua*, and 35% of the laboratories reported either the closely related *C. norvegensis* or non-*albicans* yeast. Thirty-four laboratories reported 528 susceptibility results. 22% (5/22) incorrectly reported the *C. glabrata*-isolate as fluconazole-susceptible. One laboratory incorrectly reported the *C. albicans*-isolate in specimen 2 as fluconazole-resistant.
Conclusion: The results of this third distribution of simulated clinical samples emphasise that mycological diagnosis is difficult especially in the routine and polymicrobial setting and that there is a need for the continuous training of laboratory technicians and clinical microbiologists. However, a higher proportion of laboratories succeeded this year detecting the polymicrobial nature of the blood culture and the *Aspergillus*-isolate in the presence of yeast. This suggests that quality assessment programmes including simulated clinical samples are valuable for the improvement of mycological skills in clinical laboratories.

P1350 Development of an external quality assessment scheme for antifungal susceptibility testing

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Objectives: To assess the results from clinical diagnostic laboratories to a questionnaire on antifungal susceptibility testing performed routinely and develop a suitable external quality assessment scheme to support quality assurance of testing in participating laboratories.
Methods: A questionnaire to assess the frequency and range of antifungal susceptibility testing was distributed to participants of the UK NEQAS external quality assessment schemes for Mycology and General Bacteriology. Strains distributed included *Candida parapsilosis*, *C. tropicalis*, *C. krusei*, *Rhodotorula* (*rubra*) *mucilaginosa*, *Cryptococcus neoformans* and *C. albicans*.
Results: Analysis of the questionnaire revealed that 143 of 445 respondents routinely performed antifungal susceptibility testing, most commonly for yeasts. The gradient MIC method (Etest®) and Sensititre® YeastOne™ were the most common tests used. Information from the survey was used to design the quality assessment specimens and reply forms. The level of performance with these specimens was variable with an average of 82% (range 21%–100%) of the results received for each strain/agent combination concordant with the reference laboratory results. Overall, the highest percentage of concordant results was seen for susceptibility testing with amphotericin B (93%), followed by fluconazole (89%), voriconazole (83%), flucytosine (75%) and itraconazole (71%). Participants found the *Candida krusei* the most problematic with low concordance of results with itraconazole (44%) and flucytosine (23%).
Conclusions: These data provide an interesting insight into routine testing methods in participant laboratories and overall performance with the strain/antifungal combinations. International antifungal susceptibility testing guidelines have only been validated for *Candida* species; breakpoints have yet to be established for voriconazole, moreover, some commercial methods are not validated for yeasts other than *Candida* spp. Therefore, evaluation of participant performance has to take these provisos into account. EQA plays an important role in highlighting areas of diagnostic testing where the quality of testing in clinical diagnostic laboratories can be informed by the development of international guidelines.

P1351 Development of a bioassay for posaconazole blood levels and its use in the clinical laboratory

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Objectives: Posaconazole is an extended spectrum triazole agent recently licensed for use in invasive fungal infections in patients intolerant of or refractory to standard antifungal therapy. There may be a relationship between drug exposure and the antifungal effect. We developed a bioassay method to measure posaconazole concentrations in serum, as a potential aid to optimising the therapeutic outcome of invasive fungal infections treated with this agent.
Methods: An agar diffusion method with yeast nitrogen base, glucose and sodium citrate was used with *Candida kefyr* (San Antonio strain with posaconazole MIC of 0.03 mg/L [CLSI], inoculum of 5×10^5 cfu/mL) as the indicator organism. Forty-microlitre volumes of standards (in triplicate wells), controls or patient specimens (both in duplicate) were loaded, allowed to diffuse and the plate was incubated at 37-degrees C overnight (approx. 18 h). Inhibition zone diameters were measured and used to construct an 8-point standard curve with concentrations ranging from 0.125 to 10 mg/L. Accuracy and variability (precision) was assessed against 5 controls: lower limit of quantification, very low, low, medium and high. These were analysed every day for 5 days to determine inter-assay accuracy and precision. Intra-assay variance and accuracy were determined by analysing these same controls five times within one bioassay plate.
Forty-four random serum levels were obtained from 16 patients (range 1–5 samples; 8 males; age range 18–69, 15 outpatients). The dosage was 400 mg bd in all cases.
Results: The assay was linear in the range 0.125–10.0 mg/L (r-squared values >0.99 for all runs, n = 39). Accuracies were within 10%, and intra- and inter-assay variability (precision) was <7% at 0.25, 0.4, 4 and 8 mg/L. The lower limit of quantification was 0.125 mg/L (accuracies of <6%, intra- and inter-assay variability within 3 and 10%, resp.). The limit of detection was 0.1 mg/L.
Patient results ranged from 0.63 to >10 mg/L. Concentrations (mg/L) of: <1.0 were found in 7 samples (5 patients), 1.0–2.0 in 24 samples (10 patients), 2.0–3.0 in 7 samples (7 patients), 3.0–5.0 in 5 samples (4 patients) and >10.0 in 1 sample.
Conclusions: We have developed and validated a clinically useful bioassay for posaconazole. This assay may be a valuable tool for therapeutic drug monitoring, especially in laboratories without access to HPLC or mass spectrometry methodology.

P1352 Analysis of pharmacodynamic interactions between antifungal drugs against *Aspergillus fumigatus* using the fractional inhibitory concentration index

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Background: The fractional inhibitory concentration (FIC) index is commonly used to analyse in vitro antifungal combinations. Synergy and antagonism is defined when the FIC index is ⩽0.5 and >4, respectively. However, most combinations are additive/indifferent because FIC indices range between 0.5 and 4 raising questions about the sensitivity of FIC index in detecting non-additive interactions. Because there are no in vivo studies to support these cutoffs, we studied an in vivo synergistic and antagonistic combination against an *Aspergillus fumigatus* clinical isolate (ATCC MYA-3626) with an in vitro microdilution checkerboard method and the results were analysed with the FIC index analysis.

Methods: The combinations of voriconazole + anidulafungin (VOR+ANID) and ravuconazole+amphotericin B (RAV+AMB) were tested in vivo in an experimental neutropenic rabbit model of invasive pulmonary aspergillosis using the ATCC MYA-3626. In vivo efficacy of the monotherapy and combination therapy regimens was assessed based on several outcome variables. The same combinations were tested against the ATCC MYA-3626 in triplicate with broth microdilution checkerboard technique based on the CLSI M-38A. Fungal growth was assessed spectrophotometrically at 405 nm after incubation at 37°C for 24 h and 48 h and % of growth was calculated for each drug concentration compared to the drug-free growth control. The minimal inhibitory concentration was determined for each drug alone and in combination as the lowest drug concentration corresponding to <10%. The FIC index was calculated as the MICA+B/MICA + MICB+A/MICAB where MICA and MICB are the MICs of the drugs alone and MICA+B and MICB+A are the MICs of drugs A and B in presence of the other drug, respectively.
Results: In vivo, the combination therapy of VOR+ANID was more effective than each monotherapy indicating synergistic interactions; whereas, the combination therapy of RAV+AMB was less effective than each monotherapy indicating antagonistic interactions. The FIC index of VOR+ANID was 0.31 (0.25–0.378) after 24 h indicating synergy and 0.75 (0.63–0.75) after 48 h indicating additivity. The FIC index of RAV+AMB was 2.25 (2.12–2.5) after 24 h and 1.5 (0.63–1.5) after 48 h indicating additivity.
Conclusion: Assessing antifungal drug interactions against *A. fumigatus* after 24 h may better than after 48 h to predict in vivo outcomes. The FIC index cutoff of 2 instead 4 could be used to assess antagonistic interactions.

P1353 H2S and NO affect intracellular killing of *Candida albicans* modulating GSH levels

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Candida albicans, has become a significant nosocomial pathogen. In macrophages, responsible for control of candida growth, redox status regulates the intracellular killing. Antioxidant mechanisms are likely to quench the reactive oxygen species (ROS) and to protect against any damage but, during infections, these systems are reduced. Glutathione (GSH) and nitric oxide (NO) are important mediators in various physiological and pathological conditions. Recent studies, also, show that endogenous hydrogen sulphide (H2S) could protect cells from oxidative stress by modulating ROS. In this study, we have examined the role of GSH during C. Albicans infection and the relationship between the GSH, H2S and NO in infected murine macrophages.
Methods: J774.1 cells, infected with *C. albicans*, were maintained for 1 h at 37°C, in culture medium with GSH precursors (NAC, N-acetylcysteine) or with glutathione biosynthesis inhibitors (BSO, Buthionine-sulfoximine). In addition, in infected macrophages H2S was induced with Sodium hydrosulphide (NaHS) or inhibited whit DL-propargylglycine (PAG). GSH concentration was spectrophotometrically assayed. To determine whether GSH and H2S were, also, important for the intracellular killing of *C. albicans*, infected J774A.1 were lysed and the surnatant was plated on Sabouraud dextrose agar. The number of viable candida was quantified with 10-fold dilutions spread. In infected macrophages, NO production was assessed by measuring of intracellular nitrite concentrations or by expression of inducible nitric oxide synthase (iNOS), detected by Real Time PCR.
Results: Treatment with BSO significantly reduced killing of *C. albicans* and GSH concentration. NAC addition to infected macrophages, increased GSH levels and induced a significant reduction in the viability of *C. albicans* ($P < 0.001$). NaHS caused an increase of GSH levels and a decrease of viable yeast, while PAG reduced GSH concentration and intracellular killing. In the presence of BSO, nitrite and iNOS was increased by 2-fold respect to unstimolated cells ($P < 0.001$), while NAC decreased iNOS synthesis and intracellular NO concentration. Moreover, NaHS significantly inhibited NO production, while PAG caused iNOS expression ($P < 0.001$).
Conclusions: GSH may play an important role in killing of *C. albicans* and in control of opportunistic fungal infections. We have also provided to evidence that H2S could modulate intracellular killing by increasing GSH levels and inhibiting NO synthesis.

P1354 Difference in susceptibility to hydrogen peroxide (H2O2) between *Aspergillus fumigatus* and *Fusarium solani*

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Objectives: *A. fumigatus* and *F. solani* cause life-threatening infections in immunocompromised patients. Phagocytes, particularly neutrophils and monocytes/macrophages, constitute the main innate host defences against these fungal pathogens. The oxidative antifungal pathway of NADPH oxidase-catalyzed superoxide anion (O2) production leading to hydrogen peroxide (H2O2) formation is considered critical for the phagocyte-induced hyphal damage. We previously found that *F. solani* hyphae are more resistant to phagocyte-induced hyphal damage than are hyphae of *A. fumigatus* [CMI 2005, 11(S2): 364]. Therefore, we attempted to assess the susceptibility of *A. fumigatus* and *F. solani* hyphae to the antifungal activity of H2O2.
Methods: Suspensions of the two fungi containing 10^5 conidia per ml in YNB were incubated in 96-well plates at 37ºC for 16–18 h to become hyphae. They were then incubated with 0, 50, 100 and 200 mM H2O2 at room temperature for 10 min. Each hyphal suspension was then washed with H2O twice before adding 150 microliters of a solution containing 0.25 mg/ml XTT plus 40 mg/l coenzyme Q. After incubation at 37ºC with 5% CO2 for 30 min, H2O2-induced hyphal damage was colorimetrically assessed as reduction in metabolic activity of hyphae. ANOVA with Dunnett test was used for analysis ($n = 36$).
Results: H2O2 induced a concentration-dependent hyphal damage on *A. fumigatus* ($p < 0.001$). H2O2 induced a non concentration-dependent hyphal damage on *F. solani*. The hyphal damage induced by 100 mM H2O2 on *A. fumigatus* was higher than that induced on *F. solani* (72.7% vs. 53.2%, $p = 0.048$). At 200 mM, hyphal damage induced by H2O2 on *A. fumigatus* was 85.0% as compared to 67.1% for *F. solani* ($p = 0.005$). Hyphal damage induced by H2O2 at a concentration of 50 mM on *A. fumigatus* and *F. solani* was 47.1% and 42.9%, respectively (p=ns).
Conclusion: *F. solani* hyphae are more resistant than *A. fumigatus* hyphae to H2O2, a key antifungal component of the oxidative phagocytic pathway. This finding suggests that the activities of soluble mediators of the innate immune response against medically important filamentous fungi are fungal species-dependent and may contribute to differences in susceptibility of these two pathogens to host defence.

P1355 Pathogenic fungi produce proteases to degrade cerebral immune proteins and to evade immune attack

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Objectives: Affection of the central nervous system (CNS) in the course of a fungal infection is fatal in the majority of cases. In order to determine the reasons for the incomplete elimination of invading fungi we examined the fungal capacity to degrade complement proteins and cytokines as well as functional surface molecules of immune cells in the CNS.
Methods: *Aspergillus* spp. and other pathogenic fungi were grown in medium or cerebrospinal fluid (CSF), with or without supplements. Degradation of soluble immune proteins was investigated by Western Blot. Hyphal opsonisation was examined by immunofluorescence. Cellular expression of surface proteins was quantified by FACS.
Results: The growth of *Aspergillus* spp. in CSF resulted in secretion of proteolytic factors which degraded complement proteins. The extent of the proteolysis was dependent on the time period of fungal growth and the *Aspergillus* species. *A. fumigatus*, the predominant cause of cerebral aspergillosis, induced a rather quick and strong degradation, whereas the proteolysis by *A. terreus* supernatants was weaker and rather

slow. The fungal secretion of proteases correlated with a diminished opsonisation of *Aspergillus* hyphae by complement proteins, suggesting a better protection against phagocytosis. Furthermore the proteolytic factors attack complement receptor CR3 (CD11b/CD18) and MHC on the surface of immune cells and thus interfere with efficient phagocytosis and antigen presentation. The secretion of proteases as effective immune evasion mechanism could also be detected for isolates of the *Pseudallescheria* group and for zygomycetes (*Rhizomucor pusillus*, *Rhizopus microsporus*).

In order to develop therapeutic approaches that interfere with fungal proteases and thus with immune evasion we investigated the influence of culture conditions on the secretion of proteases. The addition of various nitrogen sources to CSF prevented the secretion of these proteases. Glutamin as endogenous substance was tested in detail and was found to effectively interfere with protease secretion in a dose-dependent manner. Furthermore, first experiments using protease inhibitors showed promising results.

Conclusion: *Aspergillus* and other pathogenic fungi secrete proteases which may play a significant role in immune evasion by degrading complement and surface-bound molecules of cerebral immune cells. These proteases represent an interesting therapeutic target to decrease the lethality of cerebral aspergillosis.

P1356 Cytokine production by human polymorphonuclear leukocytes in response to different zygomycetes

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Objectives: Zygomycetes are opportunistic pathogens causing invasive infections with increased morbidity and mortality in immunocompromised patients. These infections are most frequently caused by *Rhizopus oryzae* (RO) and *Rhizopus microsporus* (RM). Infections caused by *Cunninghamella bertholletiae* (CB) are uncommon but severe. Laboratory animal studies have demonstrated that CB is more virulent than RO and RM in a pulmonary zygomycosis model. As polymorphonuclear leukocytes (PMNs) are critical components of antifungal host defence, we compared release of IL-6, IL-8 and TNF-α cytokines from human PMNs exposed to CB, RO, and RM.

Methods: 105 sporangiospores/ml of CB, RO and RM were incubated in RPMI at 37ºC for 7, 9 and 9 h, respectively, in order to yield similar biomass ranging between 0.08–0.1 optical density as measured by spectrophotometry at 450 nm. PMNs isolated from healthy adult volunteers by dextran sedimentation/ficoll centrifugation were then added at effector:target (E:T) ratio of 10:1 or 20:1. The PMN-induced cytokine release of IL-6, IL-8 and TNF-α was assessed by ELISA quantitative assay after incubation of PMNs with hyphae for 1 h. Statistical analysis ($n = 3$) was performed using ANOVA with Dunnett post-test for multiple comparisons.

Results: See the table

Treatment	Cytokines	
	IL-8	TNF-α
PMNs	30.8±3.2	0.05±0.02
PMNs+CB	575.3±55.2*	4.8±0.6*
PMNs+RO	915.2±153.7*	0.6±0.2
PMNs+RM	936.7±133.8*	1.0±0.1

*$p < 0.01$.

CB induced decreased amounts of IL-8 release from PMNs compared to RO and RM (for both $p < 0.05$), but increased amounts of TNF-alpha release compared to RO and RM (for both $p < 0.01$). IL-6 was not expressed in PMNs exposed to the three zygomycetes after 1 h.

Conclusion: CB induces higher release of TNF-α and lower release of IL-8 in PMNs compared to RO and RM, while release of IL-6 remains unaffected. These findings provide insight into the possible mechanisms of increased resistance and severity of CB infections as compared to those of other zygomycetes.

P1357 In vitro susceptibility of clinical isolates of *Dipodascus capitatus*

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Background: *D. capitatus* (teleomorph of *Geotrichum capitatum*), has been added to the lengthening list of opportunistic fungal pathogens that can cause fatal infections in inmunocompromised patients. In order to give any insight on the management of this emerging infection we have analysed the antifungal susceptibility profile of eight currently available antifungal agents against a collection of D.capitatus clinical isolates.

Methods: A total of 35 clinical strains of *D. capitatus* were included. The strains were recovered from different Spanish hospitals through a period of ten years, from 1996 to 2006, and identified by routine physiological tests. The susceptibility testing followed strictly the recommendations proposed by the Antifungal Susceptibility testing Subcommittee of the European Committee on Antibiotic Susceptibility Testing for fermentative yeast, including some modifications to allow for automation of the method and to permit the incubation period to be shortened from 72 to 48 h The antifungal agents used in the study were as follow: Amphotericin B (AMB), flucytosine (5-FC), fluconazole (FLC), itraconazole (ITC), voriconazole (VRC), ravuconazole (RVC), posaconazole (POS) and caspofungin (CAS).

Results: All isolates produced clearly visible growth after 48 hours of incubation following the method described. A wide range of MICs was observed for AMB with values ranging between 0.06 and 32 mg/L. Tree strains (3/35, 8.6%) showed a AMB MIC $\geqslant 1$ mg/L. Only one isolate was resistant in vitro to flucytosine (MIC > 32 mg/L). Fluconazole showed poor activity, with MIC $\geqslant 4$ mg/L for most of the strains tested (97%). Among new azole compounds, ITC exhibited the best activity compared with VRC, RVC and POS (Geometric Mean of MICs, 0.17, 0.22, 0.23 and 0.19 respectively). On the other hand, CAS showed poor in vitro activity against most of the strains tested (GM 24.25 mg/L)

Conclusion: (1) Isolates of *D. capitatus* were in vitro susceptible to Amphotericin B, flucytosine and new azole agents, specially ITC. (2) The majority of *D. capitatus* tested were resistant to FLC.

P1358 In vitro susceptibilities of clinical isolates of filamentous fungi to new antifungal agents

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Objectives: Anidulafungin(AND) and caspofungin (CAS), two new antifungal agents belonging to the echinocandin, act by inhibiting beta-1,3 glucan synthetase, and display a broad spectrum of action, even against isolates resistant to amphotericin B and fluconazole. A study was made of their action against filamentous fungi.

Methods: A total de 124 strains of the following filamentous fungi species were isolated fron a range of clinical samples: *Aspergillus fumigatus* (35), *Aspergillus terreus* (22), *Aspergillus flavus* (18), *Aspergillus niger* (16), *Fusarium verticilliodes* (9), *Fusarium oxysporum* (6), *Scedosporium apiospermum* (6), *Emericella nidulans* (4), *Scedosporium prolificans* (3), *Paecillomyces variotii* (3), *Fusarium solani* (2). The CLSI, (formerly NCCLS) M38-A document for antifungal susceptibility testing of filamentous fungi, broth microdilution method was used. Concentrations assayed for each antifungal agent ranged from 0.03 to 16 μg/ml. *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019 were used for quality control. MICs and minimum effective concentrations (MECs) for evaluating anidulafungin and caspofungin against moulds. Anidulafungin and caspofungin MICs($\geqslant$50% inhibition) and MECs (morfological hyphal changes) were determined.

Results: MIC90 results (μg/ml) for the two antifungal were as follows (ADN/CAS): *Aspergillus fumigatus* (2/8), *Aspergillus terreus* (2/4), *Aspergillus flavus* (2/4) *Aspergillus niger* (4/2), *Fusarium verticilliodes* (16/16), *Fusarium oxysporum* (16/8), *Scedosporium apiospermum* (2/2),

Emericella nidulans (0.5/4), *Scedosporium prolificans* (0.12/0.12), *Paecilomyces variotii* (0.06/2), *Fusarium solani* (4/2). The paradoxical effectct was more common with caspofungin than with anidulafungin.
Conclusion: Anidulafungin and caspofungin showed moderate in vitro activity against most of the species tested. Greater activity was displayed against *Scedosporium prolificans* and *Scedosporium apiospermum*. Anidulafungin was more active than caspofungin against *Aspergillus fumigatus*. *Aspergillus terreus*, *Aspergillus flavus* and *Aspergillus niger* showed little sensitivity to either antifungal agent, both of which lacked any in vitro activity agains *Fusarium verticilliodes* and *Fusarium oxysporum*.

P1359 Caspofungin Etest endpoint for *Aspergillus* shows poor essential agreement with the reference minimum effective concentration

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Objective: The minimum effective concentration (MEC) is the currently accepted broth microdilution (BMD) endpoint for measuring echinocandin activity against filamentous fungi. However, this method requires microscopic examination of fungal growth, which prevents many laboratories from adopting this methodology. We evaluated the caspofungin Etest as an alternative to the BMD for MEC determination in *Aspergillus*.
Methods: BMD was performed according to the Clinical Laboratories Standards Institute (CLSI) document M38-A using 0.016 to 32 mg/L caspofungin. MEC was determined microscopically for the first 30 specimens, correlated to the macroscopic appearance of each well, and then inferred from macroscopic appearance for remaining isolates. Etest was performed using a modification of the CLSI M44-A disk diffusion method on Mueller-Hinton agar supplemented with glucose (2%) and methylene blue (0.5 mg/L). Caspofungin Etest endpoints were measured at the intersection between *Aspergillus* growth and the Etest strip. Representative CLSI-approved quality control strains were tested to satisfy reproducibility. All tests were incubated for 24 hours.
Results: 274 clinical *Aspergillus* isolates were tested, including *A. fumigatus* (73), *A. flavus* (51), *A. niger* (90), *A. terreus* (19), and *A. nidulans* (41). The Etest endpoint was lower than BMD for 91% of clinical isolates with an overall essential agreement ($\leqslant$1 doubling dilution variance) of 18.25%. The majority of discordant Etest results varied from BMD by 2 (26%), 3 (33%), and 4 (15%) doubling dilutions, and was proportional across all species. Etest endpoints were clearly defined except for *A. flavus* and *A. terreus*, which tended to yield narrower inhibition zones with microcolony growth (disregarded). This discordance between BMD and Etest was reproducible using CLSI-approved quality control strains daily for a 10-day period.
Conclusions: Although susceptibility testing for filamentous fungi to the echinocandins is still in its infancy, the results of this study demonstrate that the caspofungin BMD MEC and Etest endpoint are not comparable measures. Unless CLSI test conditions can be optimised to reduce this disparity the caspofungin Etest cannot be used as a BMD alternative for *Aspergillus* susceptibility testing.

P1360 Clinical isolates of aspergillus are still fully susceptible to voriconazole in the POSTVORI era

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Objectives: Voriconazole (VRC, Pfizer Pharmaceutical Group, New York, NY) is the drug of choice for the treatment of invasive aspergillosis. It has proven to be very active in vitro against clinical isolates of *Aspergillus*. However, recent reports from single institutions alert to an increasing incidence in the isolation of strains of *Aspergillus* spp. with reduced susceptibility to VRC. The aim of our study was to analyse the in vitro activity of VRC against *Aspergillus* isolates collected before and after its commercialisation in 2002.
Methods: We studied 402 clinical strains of *Aspergillus* spp. The isolates were grouped into two periods: 197 strains isolated in the PREVORI period (1999–2002) and 205 strains in the POSTVORI period (2003–2007). The MICs of VRC were determined following the CLSI M-38A microdilution procedure. The MIC endpoint was defined as the lowest concentration producing complete visual inhibition of growth (MIC-0). Those strains with an MIC $\geqslant$8 mg/L for VRC were classified as susceptible according to putative breakpoints.
Results: Mean and MIC90, in mg/L, were as follows: overall (0.48/1.00), PREVORI (0.39/0.5), and POSTVORI (0.57/1.00). No VRC-resistant strains were detected in either period. However, we found that clinical strains isolated in the POSTVORI period tended to be slightly less susceptible than those from the PREVORI period ($P < 0.001$) (Figure 1).

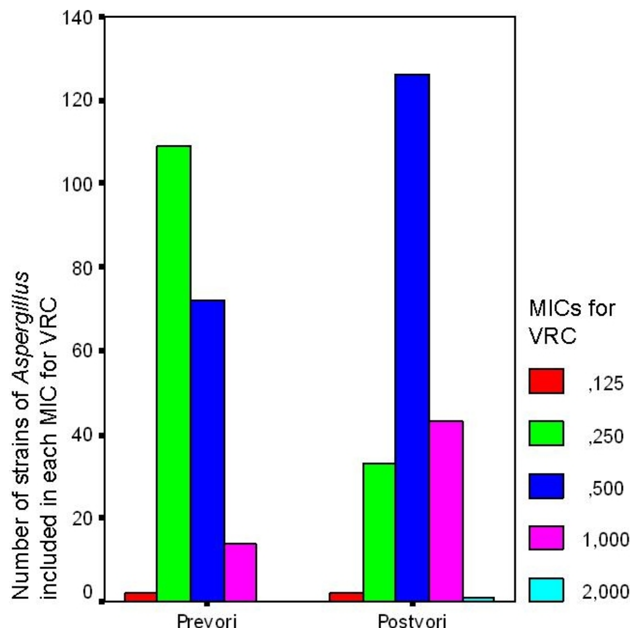

Figure 1.

During the PREVORI period, 92.9% of strains were inhibited at $\leqslant$0.5 mg/L; in contrast, during the POSTVORI period, only 78.5% were inhibited at $\leqslant$0.5 mg/L.
Conclusions: VRC remains very active against *Aspergillus* spp. clinical isolates. Although recent strains present slightly higher MICs for VRC, all strains remain fully susceptible to this agent. The clinical relevance and trend of this finding need to be demonstrated. This study was financed by grants from CIBER RES CD06/06/0058 and from FIS PI070198. Jesús Guinea Pharm D, PhD is contracted by the Fondo de Investigación Sanitaria (FIS), contract number CM05/00171.

P1361 Efficacy of combination therapy of antifungal agents against *Aspergillus*: in vitro evaluation with electron microscope

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Objectives: Combined antifungal chemotherapy has been supposed to be effective against severe *Aspergillus* infections such as invasive pulmonary aspergillosis in immunocompromised patients. However, no reliable indicators have been established to show its effectiveness in vitro and in vivo. This study focused on the evaluation of efficacy of combined antifungals by using transmission electron microscope (TEM).
Methods: We used *A. fumigatus* isolated from patient with invasive pulmonary aspergillosis. The fungal inocula and antifungal agents were prepared in accordance with M38-A recommendation of CLSI, and MICs

of amphotericin-B (AMB) and voriconazole (VOR), minimum effective concentration (MEC) of micafungin (MICA) were determined. First, based on the morphological changes under TEM, the concentrations with prominent fungicidal features (Fungicidal Activity: AC) were determined in each agent. Second, in combination of agents, checkerboard synergy tests were performed to obtain the fractional inhibitory concentration index (FIC) by examination of the changes under TEM.
Results: MIC and AC of AMB were 0.5 μg/ml, and MIC and AC of VOR were 0.5 μg/ml. MEC of MICA was ⩽0.015 μg/ml, whereas AC (disruption of the cell wall, expansion of endoplasmic reticula) was 1 μg/ml. In combination AMB with VOR, FIC was 1.0 showing additive effects. In combination MICA with VOR, FIC was 0.74(= VOR 0.06/ 0.25 + MICA 0.5/1.0) suggesting partially synergistic effects.
Conclusion: These findings suggest that this method based on the morphological changes could lead to new approaches in case that the MIC is impossible to determine such as echinocandins for *Aspergillus*.

P1362 Drug resistance modulation in yeast

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Objectives: Multidrug resistance is a defence mechanism used by cells to cope with rapidly changing growth and stress conditions, allowing them to survive in the presence of toxic compounds. In *Saccharomyces cerevisiae* and pathogenic *Candida* sp. the multidrug resistance is caused by an increased drug efflux by overexpressed membrane transporters due to gain-of-function mutations in transcription factors involved in the control of their expression. This work was aimed at the development of new strategies to reverse the drug resistance in yeast using *S. cerevisiae* and *C. albicans* as models.
Methods: Drug susceptibilities were determined by microbroth dilution method in 96-well plates according to the proposed NCCLS M27-A standard guidelines, by agar diffusion method or by spot assay. Loss-of-function pdr3 mutants in the genetic background of the *S. cerevisiae* strain ZK11−1 were selected on minimal medium containing galactose. The DNA sequence was determined with the ABI Prism 3100 DNA sequencer. Accumulation of rhodamine 6G was measured by flow cytometry.
Results: In this work we show that the susceptibility to antifungals of drug resistant and drug sensitive yeast cells can be enhanced by several ways. They involve: 1. The use of multicopy suppressors of drug resistance generated by loss-of-function mutations in genes encoding multidrug resistance transcriptional activators. 2. The inactivation of histone deacetylases associated with the chromatin remodeling. 3. The use of chemosensitising agents altering the lipid composition of yeast membranes.
Conclusion: The genetic and biochemical approaches to modulate multidrug resistance in yeast cells may prove useful in development of new strategies to combat fungal infections caused by drug resistant pathogens.

P1363 Cph1p negatively regulating MDR1 involved in drug resistance in *Candida albicans*

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Objective: The cph1/cph1 efg1/efg1 double mutant in *Candida albicans* is defective in filamentous growth and avirulent in a mouse model. Recently, we have reported that Efg1p is involved in drug resistance by negatively regulating ERG3 in *C. albicans*. The purpose of this study is to investigate the effect of CPH1 expression on the drug resistance efflux pump MDR1 in *C. albicans*.
Methods: CPH1 from *C. albicans* was cloned and expressed in *Saccharomyces cerevisiae* and *C. albicans*. The expression level of MDR1 and the susceptibilities to antifungal drugs of the transformants were determined.
Results: We have found that overexpression of CPH1 in *Saccharomyces cerevisiae* increased susceptibility to the antifungal drug fluconazole. Furthermore, mutations on CPH1 increased the expression of MDR1 as well as decreased the susceptibility to antifungal drugs in *C. albicans*.
Conclusion: Our findings indicate that though Efg1p and Cph1p may have the same effects on the virulence, they have opposite effects on drug resistance in *C. albicans*.

P1364 Efungumab shows no significant cytochrome P450 interaction in vitro

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Objectives: Efungumab (Mycograb®) is a human recombinant monoclonal antibody that inhibits extracellular heat shock protein 90. It has demonstrated efficacy for the treatment of invasive candidiasis in combination with amphotericin B. The objective of this study was to confirm that efungumab does not induce the CYP1A and CYP3A isoenzymes of cytochrome P450 using primary cultures of human hepatocytes.
Methods: Hepatocytes were isolated from tissue samples removed from living donors undergoing liver resection. Live cells were seeded into 24-well collagen-coated tissue culture plates at a density of 1×10^6 cells/ml, and once attached were incubated with serum-free media for 36 hours. Cells were subsequently incubated for 48 hours in triplicate with efungumab, the CYP1A1 inducer omeprazole (30 uM), or the CYP3A4 inducer rifampicin (50 uM). Solutions were then replaced with 0.5 ml of either 7-ethoxyresorufin (CYP1A2 probe) or [^{14}C] testosterone (CYP3A4 probe). The activities of 7-ethoxyresorufin O-deethylase and testosterone 6beta-hydroxylase were determined using standard end-point fluorimetric and radiochromatographic methods, respectively.
Results: Compared with control hepatocytes, omeprazole elicited 79.3-fold, 18.7-fold and 16.4-fold mean increases in CYP1A2 activity. The corresponding values for efungumab at concentrations of 1, 10 and 90 ug/ml were 1.6-fold, 1.1-fold and 1.6-fold, respectively. Rifampicin induced CYP3A activity by a mean of 2.3-fold, 1.7-fold and 5.5-fold over the control cells. Mean CYP3A activity was 1.1-fold, 0.9-fold and 0.9-fold over control for hepatocytes incubated with 1, 10 and 90 ug/ml efungumab, respectively.
Conclusions: Incubation with efungumab at concentrations up to 90 ug/ml did not elicit marked changes in the CYP1A2 or CYP3A4 activity of primary human hepatocytes. These data confirm that efungumab is not an inducer of these enzymes in vitro.

P1365 Pre-incubation with efungumab increases the sensitivity of *Cryptococcus neoformans* to amphotericin B and 5-flucytosine combination therapy in vitro

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Objectives: Cryptococcal meningitis is associated with poor outcome: mortality ranges from 88% to 100% in African countries. Conventional treatment is with amphotericin B (AMB) + 5-flucytosine (5-FC). 5-FC has intrinsic toxicity and the emergence of resistance is common during treatment. Efungumab (EFU; Mycograb®) is a recombinant antibody derived against the LKVIRKNIV epitope of *Candida* heat shock protein 90 (Hsp90). This study devised an assay to evaluate sensitivity of EFU pre-treated *Cryptococcus neoformans* to AMB and 5-FC in vitro.
Methods: Six isolates of *C. neoformans* were pre-incubated for 2 hours with EFU 4, 8, 16 or 32 ug/mL. Cells were washed and used to inoculate single (AMB or 5-FC) or combination (AMB+5-FC) antifungals. The MIC was determined by microtitre assay at MIC-0 and MIC-2 endpoints and Fractional Inhibitory Concentration Index (FICI) calculated. Biacore analysis was performed on a sensor chip surface covalently coated with 2 ug/ml EFU to assess binding of EFU to cryptococcal Hsp90.
Results: Before EFU pre-treatment, AMB MIC-0 ranged from 0.25 to 1 ug/mL and 5-FC MIC-2 ranged from 0.25 to 4 ug/mL. Pre-treatment with 4 ug/mL EFU reduced AMB MIC-0 range to 0.25–0.5 ug/mL, but the range of 5-FC MIC-2 was unchanged. At 8 ug/mL EFU pre-treatment, AMB MIC-0 was 0.125 ug/mL for 5 out of 6 isolates, and

5-FC MIC-2- ranged from 0.125–2 ug/mL for 4 out of 6 isolates. At 16 or 32 ug/mL EFU pre-treatment, MICs were reduced by 2–6 dilutions for each antifungal (0.0625–0.25 ug/mL for AMB (MIC-0) and 0.125–1 ug/mL for 5-FC (MIC-2)). Combination of AMB+5-FC gave lower FICIs when cells were pre-treated with EFU vs not pre-treated. FICI values decreased as EFU concentration increased (Table). Biacore analysis demonstrated the high affinity of EFU for *C. neoformans* Hsp90: K_a=1.38×10^5 M^{-1}/S^{-1}, K_d=5.53×10^{-4} S^{-1} and K_D=4.19×10^{-9} M.

C. neoformans strain	Antifungal	FICI value, Efungumab (μg/mL)				
		0	4	8	16	32
F/7844	AMB+5-FC	1	0.75	0.56	0.31	0.28
F/9002	AMB+5-FC	2	0.75	0.50	0.38	0.25
F/7854	AMB+5-FC	2	0.75	0.50	0.38	0.25
F/7986	AMB+5-FC	0.75	0.50	0.25	0.14	0.13
F/8102	AMB+5-FC	0.75	0.52	0.38	0.26	0.25
NEQAS 6035	AMB+5-FC	2	1	0.50	0.38	0.25

Conclusion: The tight-binding affinity of EFU to cryptococcal Hsp90 enabled EFU+AMB+5-FC triple therapy to be assayed in *C. neoformans*. EFU pre-treatment increased the sensitivity of *C. neoformans* to AMB, 5-FC and AMB+5-FC in vitro, suggesting potential synergy of these combinations.

P1366 *Candida albicans* biofilm drug resistance: role of CDR1 gene

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Objective: *Candida albicans* is prominent human fungal pathogen which is known to cause infections mainly in its biofilm mode of growth. Since years, azole drugs and its derivatives continue to dominate as antifungal agents against *Candida* related infections. Even though very widely acclaimed for their efficacy, these drugs are known to have side effects and limited action due chemical reaction into biofilm matrix and over expression of drug efflux pumps. In this work, we report that CDR1 gene of *C. albicans* is responsible for enhanced drug resistance in biofilm mode of growth.
Methods: The *S. cerevisiae* strain used was AD1-8u- as cloning host and Apcdr1 was its derivative expressing *Candida* drug resistance protein (Cdr1p). Fluconazole commonly used against *Candida* infections was chosen as a positive control. Biofilm quantification was done by XTT (2,3-bis[2-Methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide) reduction assay.
Results: Characterisation of the clone Apcdr1 showed expression of CDR1 gene inside *S. cerevisiae* host AD1–8u-. Data showed that Apcdr1 was about 48 times resistant than AD1-8u- for fluconazole, while in biofilm mode of growth this resistance was increased to 73 times for the same drug.
Conclusions: It is encouraging to note that CDR1 gene is responsible for *C. albicans* biofilm associated drug resistance and overexpressed during biofilm formation.

P1367 Biofilm-forming capacity and biofilm antifungal drug resistance of *Candida* bloodstream isolates

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Objective: Candidaemia is one of the most important nosocomial bloodstream infections. Biofilm (BF) formation is considered a virulence factor responsible for catheter-related candidaemia. Our aim was to investigate the capacity of bloodstream *Candida albicans* (CA) and *Candida parapsilosis* (CP) isolates to form BF and to correlate the degree of BF formation with the resistance of BF to voriconazole (VRC) and posaconazole (PSC).
Methods: 5×10^5 planktonic (PL) cells/mL were grown in YNB medium with 2% glucose at 37°C for 24 h. For biofilm formation, 10^6 cells/mL were grown on silicone disks placed at the bottom of 96-well plates in RPMI-1640 under constant shaking at 37°C for 48–72 h. BF production was then evaluated by XTT metabolic assay, safranin staining and light microscopy (LM). Documented BF producers (CA-M61 and CP/PA71) were used as positive controls (metabolic activity by XTT assay: 100%). Isolates that a) showed XTT conversion ⩾80% of positive controls, b) stained with safranin and c) produced microscopically a dense network of fungal elements were considered high BF producers. XTT conversion of <80% defined non BF producers, while conversion ⩾80% with inconsistent safranin and LM findings defined low BF producers. PL MICs of VRC and PSC were assessed by CLSI M27-A2 method. BF MICs were determined as the minimum antifungal concentration causing ⩾50% reduction in the metabolic activity of BF as compared to drug-free controls. All isolates were tested in triplicate at 3 different experiments.
Results: A total of 43 bloodstream *Candida* isolates (29 CA and 14 CP) were examined. BF production was detected in 89% of CA isolates vs. 43% of CP ($p < 0.01$). Among CA isolates, 72% and 17% were high and low BF producers, respectively; among CP isolates, the high and low BF producers were 36% and 7%. PL MIC90 (mg/L) of VRC was 0.037 and of PSC 0.063 for CA isolates, while they were 0.031 and 0.063 for CP isolates, respectively. BF MIC90 of both VRC and PSC were 1024 mg/L for CA and CP. No correlation was observed between BF-forming capacity and BF azole MIC pattern.
Conclusion: BF-forming capacity is a frequent characteristic especially among CA clinical isolates. Both high and low BF producers exhibited high MIC to azoles, which may account for treatment failures with these agents in BF-related bloodstream infections.

P1368 Adherence and biofilms of *Candida* species on catheters. Quantification and comparison of the two measures

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Objective: comparing adherence and ability to produce biofilms of *Candida* species on the surface of catheters expressed in colony forming units per ml.
Methods: 79 clinically isolates of yeasts from genus *Candida*: 23 *C. albicans*, 23 *C. parapsilosis*, 17 *C. glabrata* and 16 *C. tropicalis* were employed on catheters of three different synthetic material: polyurethane, polyvinyl chloride (PVC) and Teflon. To measure the adherence catheters were cut into 1 cm segments and were incubated together with an adjusted inoculum of yeasts (optical density of 1.0 at 540 nm wavelength). The incubation was 35°C for 90 minutes to establish baseline adherence and further 72 hours to assess the biofilm production. The catheter was removed, placed in 2 ml of PBS and vortexed at maximum speed. This suspension was used to determine the biofilm production counting the viable colonies expressed in ufc per ml. The results were then transformed into neperian logarithm and corrected according to the contact surface of the biomaterial.
Results: in adherence measures the four species presented the highest values on PVC. Significant differences were obtained with *C. albicans*, *C. tropicalis* and *C. glabrata* when comparing PVC and Teflon. Differences between PVC and polyurethane were also obtained with *C. albicans*, *C. parapsilosis* and *C. glabrata* and in the case of *C. tropicalis* differences there were also differences between polyurethane and Teflon.
Biofilms: *C. albicans*, *C. parapsilosis* and *C. tropicalis* presented the highest biofilm formation on Teflon. Statistically significant differences in the ability to produce biofilms of the three species were obtained when comparing Teflon with PVC and with polyurethane. But differences between polyurethane and PVC were only obtained with *C. tropicalis*. The greatest biofilm formation was obtained on PVC with *C. glabrata* but without showing significant differences between the three biomaterials.
Conclusion: in adherence measure they all presented a higher adherence on PVC. There were significant differences among the materials. In the

biofilm formation measure, however, all except for *C. glabrata* showed a greater production on Teflon. Adherence and biofilm formation vary depending on the catheter used and on the measuring time.

P1369 Relationships between *Candida albicans* and free-living amoebae

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Objectives: Dental units water may become heavily contaminated by various organisms: free living amoebae, *Candida* sp, *Legionella pneumophila*, *Pseudomonas aeruginosa*, ... Patients and dental staff can be exposed to water and generated aerosols. The objective of this study was to explore the relationships between *Candida albicans* and *Hartmannella vermiformis* or *Acanthamoeba castellanii* potentially recovered from dental units water.
Methods: *A. castellanii* (ATCC 30234) or *H. vermiformis* (ATCC 50256) trophozoites were incubated with *C. albicans* blastospores in PBS at 27°C to give a final multiplicity of infection of 0.1. After 2, 4 or 6 h of contact, a microscopical examination of the cocultivation was realised in order to verify the potential ingestion of blastospores by amoebae. After 24, 48 or 72 h of cocultivation, lysates obtained from cocultures were plated on Sabouraud agar to count CFU.
Trophozoites ($5{\cdot}10^5$/ml) of each strain of amoebae were incubated during 24, 48 or 72 h in PBS at 27°C. Amoebae were then pelleted and *C. albicans* blastospores ($5{\cdot}10^4$/ml) were cultivated at 27°C during 24, 48 or 72 h in the resultant supernatant. After incubation, each tube content was plated on Sabouraud agar plates to count CFU.
Results: In cocultivation experiments, the presence of *A. castellanii* induced a decrease in *C. albicans* growth, compared to fungal growth in PBS. On the contrary, *H. vermiformis* allowed an increase in *C. albicans* growth whatever the duration of incubation. Moreover, the microscopic examination of cocultures showed a *C. albicans* internalisation only by *A. castellanii*.
The only contact with *H. vermiformis* supernatants led to a number of fungal CFU increase, particularly noticeable with the 72 h supernatant.
Conclusion: In this preliminary study, *A. castellanii* and *H. vermiformis* in contact with *C. albicans* had two opposite comportments. *A. castellanii* was able to phagocyte and destroy *C. albicans*. At the opposite, *H. vermiformis* seemed able to release in the medium substances capable to increase *C. albicans* growth. Further studies have to be conducted to explain these differences.

Diagnostic bacteriology

P1370 Evaluation of the automated urine culture system Alfred60 for the screening of significant bacteriuria

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Objectives: Analysis of urine samples is the most prescribed bacterial diagnostic test in clinical laboratory. We aimed to evaluate the ability of the new automated incubation system Alfred60 (Alifax, Italy), compared to classic solid media culture, to screen for samples with significant bacterial growth which would be submitted for manual culture.
Methods: 1025 mid-stream urine samples collected from hospitalised and ambulatory patients within a one-month period were plated onto a chromogenic agar Uriselect4 (Uri4, Biorad) using calibrated loop and transferred into the Alfred60 system for automated testing. A turbidity-based growth curve generated by Alfred60 for each sample was converted to an approximate bacterial quantification in CFU/ml. A sample was reported as positive by Alfred60 if it displayed a bacterial count of ⩽10000 CFU/ml. By Uri4, urine samples were classified taking account the bacterial growth quantification, the number of species and the presence of the contamination flora. A sample was considered positive by Uri4 after interpretation applying the Kass criteria. Samples reported as negative by Alfred60 and positive by Uri4 underwent further discrepancy analysis including reviews of urinary leucocytes count and patient clinical data.
Results: Of the 1025 urine samples tested, 137 were classified as positives (13%) and 733 negatives (72%) by both methods, representing a total agreement of 85%. 136 samples (13%) were positive by Alfred60 and negative by Uri4 while 19 (2%) were negative by Alfred and positive by Uri4. Of these 19 major discrepancies, 8 bacteriuria were considered clinically insignificant, 7 clinically relevant and the signification remain unknown for 3 patients. Positive and negative predictive values after discrepancy analysis were 50% and 99% respectively.
Conclusion: Alfred60 is a promising urine screening system which can correctly and quickly (5 h) render results as negative for the majority (73%) of urine cultures requested and therefore save as many unnecessary solid culture media. However, Alfred60 positive samples still require further confirmation by classic culture method with possible identification and susceptibility testing of pathogens.

P1371 Clinical evaluation of a preservative impregnated sponge to stabilise urine samples transported for microbiological culture

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Objective: Evaluation of the clinical ability of a preservative impregnated sponge to preserve the quantity and quality of bacterial pathogens in urine during transport.
Methods: 935 urines collected for culture were poured over a routine culture paddle (Starplex Dip N' Count). The preservative sponge (Uriswab, Copan Italia) was then dipped into the remaining urine to absorb the sample (approx. 5s). The culture paddle was incubated 18 to 24 h and read according to standard quantitation protocols. Urine from the sponge was cultured by squeezing the tube to express the urine; and with a 0.001 ml calibrated loop, the expressed urine was plated on blood and MacConkey agar. The plates were incubated 18–24 h and read according to standard quantitation protocols. Significant cultures were considered as those that contained urinary tract pathogens at amounts considered to reflect probable urinary infection. Urine was also sampled multiple times from the Uriswab to investigate any quantitative difference in sampling urine from the sponge over time.
Results: 843 of the cultures (90%) yielded equivalent results for quantitation (± one log10) and micro-organism isolated. There were 92 (9.8%) discrepant results between the two systems where the quantitative difference was greater than one log10; 29 (3.1%) had potential pathogens isolated. Of these, 10 were the same organism with the culture paddle (4) or Uriswab (6) having the higher count; 13 did not grow from the sponge and the remainder showed various discrepancies. 7 of 17 cultures grew at significant quantities from the dipslide only while the remaining 10 grew significant pathogens from the Uriswab only. The remaining 63 discrepant results (6.7%) were not clinically significant: 34 of the 63 were lactobacilli or diphtheroids that did not grow from the preservative sponge. Multiple sampling over time did not alter the quantitation from the sponge cultures
Conclusions: The two systems agreed in 90% of cases. Quantitative variation observed in a few cultures may be due to sampling error. The preservative sponge appeared to reduce the growth of common contaminants such as lactobacilli and diphtheroids. The clinical utility of this observation remains to be determined. The preservative sponge offers a simple, accurate and stable alternative for transport of urine for microbial culture.

P1372 A comparison of self-collected nasal flocked swabs with staff-collected nasopharyngeal or nasal swabs for respiratory tract sampling in volunteers

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Objectives: Nasal swabs (NS) for diagnosis of respiratory viruses are less invasive than nasopharyngeal swabs (NPS), and may enable self-collection. Previously, we studied 2 NS prototypes, and found them

equivalent to NPS in sampling epithelial cells, but self-collection was inferior to staff collection. In this study we modified one of the nasal flocked swab (NFS) to optimise sampling and comfort, and to validate that 2 sequential NS will optimise respiratory cell sampling. Our objective was to examine if the new Copan NFS is equivalent to NPS in sampling the respiratory tract, and if self-sampling is equivalent to staff sampling.
Methods: 55 volunteers had 2 self-administered NFS, followed by 2 staff-administered NS using NFS or rayon swabs in random order. Pictorial instructions were provided. Discomfort, ease of administration, and preferences were assessed. The 2nd self-collected swab was compared with the staff-collected swab. 20 subjects had 2 NPS with pernasal FS or rayon swab. Swabs were placed in a one mL tube of UTM; 500 ul was used for nucleic acid extraction and 500 ul to prepare cell smears. Epithelial cells were counted under an UV microscope; Averaging 4 fields or 10 fields when less than 10 cells per high-powered field (hpf) were present. DNA was quantitated using a beta-Actin real time PCR.
Results: In the 55 volunteers, the 2nd self-collected NS was superior to the initial swab, with a mean (SD) of 117 (65) vs. 67 (43) cells/hpf. The 2nd self-collected NS was superior to staff-collected rayon NS {38 (25) cells/hpf}, and comparable to staff-collected flocked NS {132 (56) cells/hpf}. In the 20 subjects with NPS, the mean NPS (SD) cell yields were 145 (43) and 55 (30) for the flocked and rayon, respectively; and 136 (53) and 32 (22) for flocked or rayon staff-administered NS. A high correlation was found between the cell count and log DNA copies/ml ($R=0.9$, $P<0.001$). No difference was found between self and staff administered flocked NS or NPS. Flocked NS performed better than rayon with significant higher mean log DNA count. Mild discomfort or ease in self-swabbing was reported. Self-swabbing was preferred to staff collection.
Conclusions: The new Copan nasal flocked swab design is superior to rayon NPS or NS, and equivalent to flocked NPS, for sampling respiratory epithelial cells or DNA. Self nasal sampling is feasible and easy to perform, and equivalent to staff sampling. 2 sequential swabs are required for optimal cell yield.

P1373 European external quality assurance for the laboratory diagnosis of diphtheria

S. Neal, A. Efstratiou on behalf of DIPNET

Objectives: The diphtheria surveillance network (DIPNET) is officially recognised as a Dedicated Surveillance Network (DSN) and now encompasses 25 European countries. A key DIPNET objective is to assess microbiological procedures for diphtheria in order to harmonise methods and laboratory performance across the EU.
Methods: A questionnaire requesting detailed information on the level of reference services offered, methods for identification and toxigenicity testing, was sent to 25 countries. A panel of six simulated throat specimens were prepared and distributed to all countries in September 2007. Participants were asked to isolate, identify and perform toxigenicity testing on any corynebacteria present.
Results: Completed questionnaires and results for the six simulated specimens were submitted to the coordinating centre in London. Seventeen participants stated they are designated National Diphtheria Reference Centres with the UK being the only WHO Collaborating Centre. A variety of tests were used for screening and identification of corynebacteria, including the cystinase test (11/25), pyrazinamidase test (12/25) and commercial kits (19/25). Most used the classic Elek test for toxigenicity testing (18/25) with variations in serum sources and antitoxin concentration. Many laboratories reported problems obtaining reagents and media for these specialised tests.
The reported species were concordant with the intended EQA results in all centres for 3/6 specimens (EQA07–1, *Corynebacterium diphtheriae*; EQA07–2, *C. diphtheriae*; EQA07–6, *C. pseudodiphtheriticum*). However, concordance was lower for EQA07–3 (*C. diphtheriae*, 11/25 centres), EQA07–4 (*C. ulcerans*, 21/25) and EQA07–5 (*C. diphtheriae*, 18/25). Biotype results also revealed discrepancies; from 7/25 matched for EQA07–5 (var. *intermedius*) to 24/25 for EQA07–2 (var. *gravis*). Alarmingly, the intended toxigenic results were reported as non-toxigenic or not performed by four and eight centres (for EQA07–2 and EQA07–4, respectively). Further detailed results will be presented.
Conclusions: Various methods and practices are performed for identification and toxigenicity testing of corynebacteria across Europe. The discrepancies in this study alarmingly reflect the complacency and minimal awareness that still exists for diphtheria diagnostics. Significant efforts will be made by DIPNET to harmonise methods through training workshops, EQA studies and revision of the WHO manual for the laboratory diagnosis of diphtheria.

P1374 Use of quality indicators by clinical bacteriology laboratories in Ontario, Canada – External quality assessment survey by Quality Management Program–Laboratory Services

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Objective: To assess current practice on the use of quality indicators (QIs) by bacteriology labs in Ontario.
Methods: Quality Management Program–Laboratory Services is a mandatory external quality assessment (EQA) and accreditation program for clinical labs in Ontario. As part of the microbiology EQA program, the 104 Ontario labs licensed for bacteriology in June 2007 were required to complete a questionnaire on QIs monitored in the past five years and categorise each QI as pre-analytic, analytic or post-analytic. Labs occasionally incorrectly categorised the phase of the testing process being monitored and these results were appropriately reclassified. For those QIs monitored in the previous 12 months, labs were asked to provide details on data collection, target values and whether these had changed over time, results of monitoring and corrective actions undertaken.
Results: The 104 labs reported monitoring a total of 947 QIs over the five years (mean: 9; median: 9; range: 0–22). Specimen rejection rate ($n=31$), blood culture contamination rate ($n=38$), blood culture volume ($n=35$), transit times ($n=31$) and number of blood culture sets ($n=39$) were the most common pre-analytic QIs. Results review prior to report release ($n=35$) and correlation of Gram stain results with culture ($n=3$) were the most frequently reported analytic QIs. Report turnaround time (TAT) for CSF ($n=39$) and blood culture ($n=30$) Gram stains and review of reported results for transcription errors ($n=36$) were the most common post-analytic QIs. 125/947 (13%) of the QIs reported by labs were deemed to be erroneous QIs (e.g. quality control, temperature monitoring of equipment). Labs reported compiling data manually for most QIs. Not all labs had established target values for their QIs and some of the values reported were not well-defined (e.g. reduce specimen rejection). Only 6% of the target values had been revised over time. Corrective actions implemented as a result of monitoring QIs were variable and not all were appropriate.
Conclusion: The use of QIs by bacteriology labs is widespread in Ontario. Nevertheless, this survey identified a lack of understanding of QIs by some labs as evidenced by misidentifying basic QC as a QI, lack of targets or thresholds for some QIs and the need to revise targets over time in order to effect process improvement. QMP–LS released a summary of these findings to Ontario labs along with educational commentary to assist labs in improving their quality systems.

P1375 Evaluation of three immunochromatographic assays for detection of *Legionella pneumophila* serogroup 1 antigen in urine

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Objective: The aim of our study was to evaluate three immunochromatographic (ICT) urine antigen tests (Binax NOW *Legionella* antigen test, SAS *Legionella* antigen test and Trinity Biotech Uni-Gold *Legionella* antigen test) for the detection of the antigen of *Legionella pneumophila* serogroup 1 in frozen urine samples.

Methods: Urine samples were collected in 2001 during the massive outbreak of Legionnaires' disease (LD) in Murcia (Spain) and were stored at –80°C until processing was performed. We included 52 urine samples from 52 patients with LD (cases). A case of LD was defined as a patient with pneumonia who had radiological signs of infiltration and who showed laboratory evidence of infection with *L. pneumophila* by a positive serology. This group of 52 samples was divided in two, one of them with Binax NOW positive urinary antigen in 2001 (29 nonconcentrated samples, 9 concentrated samples; positive control) and the other with Binax NOW negative urinary antigen in 2001 (10 nonconcentrated samples, 4 concentrated samples; negative control). The three test were performed simultaneously and the results were calculated according to the manufacturers' instructions.
Results: We compared, six years later, in 2007, the sensitivities and specificities of these three assays. The sensitivities and specificities are shown in the table.

	Nonconcentrated samples			Concentrated samples		
	Binax NOW	SAS	Uni-Gold	Binax NOW	SAS	Uni-Gold
Sensitivity	100% (29/29)	87.9% (25/29)	55.2% (16/29)	66.7% (6/9)	55.6% (5/9)	33.4% (3/9)
Specificity	100% (10/10)	100% (10/10)	100% (10/10)	100% (4/4)	100% (4/4)	100% (4/4)

Conclusion: The Binax NOW urinary antigen test is superior to the SAS *Legionella* test and the Biotech Uni-Gold test for the diagnosis of infections caused by *L. pneumophila* serogroup 1. As demonstrated here, the stability of the urine antigen of *L. pneumophila* serogroup 1 remains acceptable overtime.

P1376 Value of Gram stain and quantitative culture of washed sputum samples in patients with lower respiratory tract infections

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Objectives: Many microorganisms cause lower respiratory tract infections (LRTI) but approximately in half of the cases aetiological agent cannot be determined. We analysed the value of Gram smear examination and quantitative culture of sputum samples for the diagnosis of LRTI.
Methods: A portion of the sputum was used to make the initial Gram smear and samples contain more than 10 squamous epithelial cells per low power field(LPF), were not further processed. Quality of the sputum was compared to results obtained using a rapid strip test (Combur test; Roche Diagnostics, Switzerland). Acceptable specimens were first inoculated into culture plates and than homogenised with physiological serum, vortexed, centrifuged, and incubated for 15 minutes after adding N-acetyl-L-cysteine. A second Gram smear was made and 1:10 dilution of washed sample was inoculated culture plates for incubation. Positivity threshold was >106 cfu /ml and bacteria were identified by using standard procedures.
Results: During a one year period (July 2006-July 2007), 620 sputum samples taken from 489 patients with a preliminary diagnosis of LRTI were studied. More then half of the samples (55.3%) were not acceptable using the number of epithelial cells as rejection criteria and in only 22.1% of samples there were more than 25 leukocytes per LPF. Using a standard gravity threshold of >1.01, the sensitivity of the strip was 70% and the specificity was 74%. We detected an aetiological agent in 63.5% of acceptable specimens by qualitative method, whereas this rate was 52% for qualitative method ($p < 0.05$). *Haemophilus influenzae* was the most common isolated pathogen, followed by Gram-negative bacilli (*Pseudomonas* spp., *Escherichia coli*, and Enterobactericae spp.) and *Streptococcus pneumoniae*, respectively. The overall sensitivity and specificity of Gram staining was 78.6 and 82%, whereas specificity was reached to 100% for *H. influenzae* and *S. pneumoniae*. Although statistically insignificant, Gram-negative bacteria were very clearly seen in washed smears. We believe that interpretation of those samples would be much easier for inexperienced personnel.
Conclusion: Quantitative culture method for sputum sample is superior to qualitative method and advisable for routine laboratory. Although there are contradictory results in the literature about the Gram examination of sputum smear, appeorently skilled interpreters will give valuable data for the empirical therapy of LRTI.

P1377 Development of a new test panel for identification of both Gram-negative and Gram-positive bacteria

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The objective of this project was to simplify biochemical/phenotypic testing of bacteria by developing a single test panel that could be used to identify both Gram-negative and Gram-positive bacteria. This has been impossible in the past because bacterial genera are quite diverse and no one has found universal culture and chromogenic testing chemistries that would work with a broad range of genera. We employed Phenotype MicroArrays to study, in detail, the metabolic and chemical sensitivity properties of diverse bacteria. From this effort we succeeded in developing assay conditions and a tetrazolium-based colorimetric chemistry that can be used to fingerprint and identify more than 1,000 species, including Gram-negative enteric, non-fermenter, and fastidious species as well as important Gram-positive species such as *Bacillus*, *Corynebacterium*, *Staphylococcus*, *Streptococcus*, *Lactobacillus*, and *Nocardia*. Using computer-assisted analysis we selected from the Phenotype MicroArray assays a final set of 94 tests that are taxonomically informative. The 71 carbon source utilisation assays include sugars, carbohydrates, sugar alcohols, hexose-phosphates, hexuronic acids, D- and L-amino acids, carboxylic acids, and other types of naturally-occurring biochemicals. The 23 chemical sensitivity assays test the sensitivity to various potentially-inhibitory conditions. The conditions include high salt (8% NaCl), high lactic acid (1%), low pH (5.0), and sensitivity to other taxonomically useful agents such as antibiotics, cations, anions, oxidisers, and detergents. Because the panel identifies both Gram-negative and Gram-positive species, a bacterial isolate can be identified without a Gram-stain or any other pre-tests. The one-minute set up procedure consists of preparing a cell suspension from agar-grown cells, and inoculating 100 μl into each well of the panel. The system recommends 33°C for incubation, which permits identification of many more species. In addition to simplicity, a major advantage over identification based on 16s-rDNA gene sequence is that you simultaneously gain detailed information about the properties and growth phenotypes of the bacterium that you have identified.

P1378 Evaluation of a rapid antigen detection test for the diagnosis of Streptococcal pharyngitis in children and its impact on antibiotic prescribing

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Objective: To study the performance of Becton-Dckinson Link 2 Strept A Rapid Test (RADT) in the diagnosis of streptococcal pharyngitis in children presenting with upper respiratory tract symptoms and fever and its impact on antibiotic prescribing
Methods: The study was a prospective non-randomised study in which private-practice and nosocomial paediatricians participated. Children 2–14 years old presenting with fever and at least one of: tender anterior cervical lymh nodes, tonsillar exudate or redness and absence of cough were enrolled in the study. Padiatricians were assigned in two groups: Group A which included only private-practice paediatricians who managed children only on clinical grounds and according to their usual clinical practice. They only had to fill a form with demographic data and information about the child's illness, their diagnosis and antibiotic prescribing. Group B consistent of private-practice (B1) and of

nosocomial paediatricians (B2) who performed the RADT and a throat culture. Antibiotic prescribing in this group was guided by a positive RADT or culture. A similar data form was also filled as in group A.

Results: During the 2-year study period, 602 children were evaluated. Streptococcal pharyngitis was diagnosed by RADT and/or culture in 119 (36.6%) of the 325 children tested. The sensitivity, specificity, positive and negative predictive values of the RADT were 83.3%, 90.55, 83.8% and 93.3% respectively. A stepwise increase of Radt sensitivity was noted among children with 2, 3, or 4 clinical criteria (80.6% to 95.8%). By logistic regression analysis tender anterior cervical nodes and absence of cough were found to predict a positive throat culture (OR=4.381, CIs=1.106–17.355). Paediatricians of group A prescribed more frequently antibiotics compared to those of group B(82.3% versus 32.6%, $p < 0.001$). During the study no streptococcal pharyngitis related complications were noted and only one child presented with a rash as an allergic reaction to a cephalosporin

Conclusion: RADT presents with a reliable accuracy in the diagnosis of streptococcal pharyngitis in children, used in private practice and in the hospital. It is also associated with a significant reduction in antibiotic prescribing when used to guide therapy in children presenting with symptoms of pharyngitis. When only clinical criteria can are used, tender cervical lymph nodes and the absence of cough may predict streptococcal pharyngitis and guide therapy.

P1379 VIDAS *C. difficile* toxin A&B assay performance evaluation and epidemiological analysis of samples collected in UK and US

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Objective: Evaluate the performance for the VIDAS *C. difficile* Toxin A&B test (CDAB): sensitivity and specificity compared to the Gold Standard Cytotoxicity Assay (CTA) and assay precision. Positive samples collected were further identified by ribotyping.

Methods: Precision was determined at 3 sites: the 6 samples were tested in duplicate, in 2 runs per day, with each of 2 VIDAS CDAB reagent lots in 3 sites over a period of 6 days. Sensitivity and specificity were determined during a clinical trial where over 1000 samples were collected from 2 sites, one in the US and one in UK in order to have a representative collection of 100 positive samples. Fresh stool sent to the laboratory for suspicion of diarrhoea due to *C. difficile* were tested in parallel with VIDAS CDAB and CTA assays. ROC curve analysis was performed to validate the cut-off established during the development of the assay. *C. difficile* strains were isolated from the positive samples and submitted for PCR ribotyping and identification.

Results: Analysis of the precision study results show the CV's for total precision are less than 12% for all panel members excepted for the negative sample. Sensitivity of VIDAS CDAB compared to CTA was 88.3%. Specificity of VIDAS CDAB compared to CTA was 99.8%. The PPV was 98.1% and the NPV was 98.4%. The ROC curve analysis confirmed the choice of the assay cut-off and equivocal zone. Ribotyping results of over 87 positive samples are presented according to their geographical origins: ribotype 017(2%), ribotype 027(30%), ribotype 106(16%).

Conclusion: The total precision for the VIDAS CDAB test was <12% and indicate a precise pre-analytical stool processing method. The sensitivity and specificity of the VIDAS CDAB test in comparison to CTA was 88.3% and 99.8% respectively. The VIDAS CDAB test has the ability to detect different strains of *C. difficile*, including the strains 017 and 027, which are very important for the epidemiology and treatment of *C. difficile* associated disease.

P1380 Evaluation of three single-use immunoassays for toxins A and B in the diagnosis of *Clostridium difficile* associated diarrhoea

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Background: *C. difficile* (CD) causes toxin-mediated gastro-intestinal diseases and is considered as a major nosocomial enteropathogen. So far, the gold standard for the diagnosis of CD associated disease (CDAD) is the cytotoxicity assay (CTA). But, since 2006, the use of a rapid test detecting both toxins A and B is recommended for the diagnosis of CDAD. We evaluated three single-use immunoassays availables in France.

Methods: Only consecutive stool specimens from hospitalised adults with suspected CDAD were included in the study. The purpose of this evaluation was to compare tree rapid single-use enzyme immunoassays (ImmunoCard® Toxins A&B) (Meridian Bioscience Europe) (ICTAB) ToxA/B Quik Chek® (BMD, France) (QCHEK), Xpect® (Oxoid, France) (XPECT) to an in-house cytotoxicity assay using MRC5-cell culture for detection of CD in clinical stool specimens. A toxigenic culture (TC) using TCCA plates (Taurocholate, Cycloserin, Cefoxitin, Agar) was also systematically performed. All stools were blindly tested simultaneously and the results were read independently by two persons. For each test, the intensity of a positive test was noted.

Results: A total of 200 fresh specimens were tested according to the manufacturer's instructions. Of these, 30 specimens (15%) were positive by CTA and 36 (18%) by TC. As compared to CTA, the ICTAB, QCHEK and XPECT tests have respectively an overall sensitivity of 80, 80 and 53% and a specificity of 98, 95 and 98% and correlation of 96, 93 and 91% were observed. There was only one discrepant result resolved by toxigenic culture of which CTA was negative and ICTAB and QCHEK tests positive. Among true and false positive results of the tests, a faint intensity of colouration was observed for 17 and 67%, 33 and 100% and 25 and 100% respectively for ICTAB, QCHEK and XPECT tests.

Conclusion: Cytotoxicity assay remains the most specific and sensitive assay for the detection of pathogenic CD but the ICTAB and QCHEK could be considered as valuable alternative test with sensitivity higher than XPECT. Otherwise, more false positive results were observed with QCHEK.

P1381 Evaluation of the Uni-Gold™ *Legionella* Urinary Antigen Test, a new immunochromatographic test in comparison with the Binax NOW test

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Objectives: Evaluation of the Uni-Gold™ *Legionella* Urinary Antigen Test (Trinity Biotech, Wicklow Ireland) sensitivity and specificity by using both fresh and retained frozen samples, in comparison with the Binax NOW *Legionella* test (Binax, Portland, ME).

Methods: 120 prospective fresh urine samples submitted to our laboratory for *Legionella* urinary antigen detection and 30 repository urine samples from 30 patients with known legionnaires' disease collected between 2004 and 2006 and stored at −20°C until processing, were tested. Evidence of infection with *L. pneumophila* was established on the detection of urinary antigen with the Binax Now *Legionella* test and on clinical and/or radiological argument of pneumonia.

Concentrated and unconcentrated urines samples were tested by the Uni-Gold test and compared to the results of concentrated urines tested simultaneously by the Binax Now *Legionella* Urinary Antigen test. Concentration of urine samples was performed by centrifugal ultrafiltration (Amicon Ultra-4; Millipore Corporation, Bedford, Mass). All samples yielding discordant results were tested by the Biotest EIA reagent (Biotest, Dreieich, Germany)

Results: On the 120 fresh urine samples, 119 samples urine showed identical results with the Uni-Gold test and Binax Now *Legionella* test including 111 negative and 8 positive urine samples. The results

were identical for non-concentrated and concentrated urine samples. The one urine sample yielding a discrepant result between the two tests was confirmed as *Legionella* urinary antigen negative with the Biotest EIA reagent, but positive with the Uni-Gold test only; it was collected from a patient without pneumonia. The 8 patients with positive urine samples were confirmed clinically to have legionnaires' disease. Thus the specificity of the Uni-Gold test was 99.1% compared to 100% for the Binax Now test

On the 30 repository urine samples, 29 and 25 yielded positive Uni-Gold test results for concentrated and unconcentrated urine samples respectively and 30 yielded positive Binax Now test result for concentrated urine. Established on the 38 true positive urines samples, the sensitivities of the Uni-Gold test were 97.3 and 86.8% for concentrated and unconcentrated urine respectively.

Conclusion: The Uni-Gold™ *Legionella* Urinary Antigen Test was found to be a valuable test for the rapid detection of *Legionella pneumophila* serogroup 1 antigen in concentrated urines.

P1382 Impact of rapid identification of *C. albicans* and *C. glabrata* directly from blood cultures using PNA FISH technology on selection of antifungal therapy

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Objectives: We studied the performance of the peptide nucleic acid fluorescent in situ hybridisation (PNA FISH) assay for rapid identification of *Candida albicans* and *Candida glabrata* directly from blood cultures within hours, compared to routine methods with time to results at 1 to 2 days. The impact of differentiating these 2 species in real time on the selection of or change to the most effective antifungal treatment (Tx) was evaluated. Our 2007 antibiogram demonstrated that fluconazole (FLU) susceptibility for isolates of *C. albicans* and *C. parapsilosis* was nearly 100%; FLU resistance was 21%, 50% and 10%, for *C. glabrata*, *C. krusei* and *C. tropicalis*, respectively. The emerging resistance to FLU in non-*albicans* strains and their predictable susceptibility to caspofungin (CP) often results in empiric Tx with CP.

Methods: 37 blood cultures newly Gram stain positive for yeast were evaluated for the presence of either *C. albicans* or *C. glabrata* or neither by PNA FISH (AdvanDx, Woburn, MA) technology. The assay uses fluorophore-labeled dual probes with staining green for *C. albicans* and red for *C. glabrata*. Routine identification was performed using MicroScan panels (Dade Behring, Sacramento CA) and CHROMagar selective media (BD, Sparks, MD).

Results: The *Candida* spp. recovered included 15 *C. glabrata*, 10 *C. albicans*, 5 *C. parapsilosis*, 2 *C. tropicalis*, 1 *C. krusei*, and 1 *Candida dubliniensis*. The sensitivity, specificity, positive and negative predictive values of PNA FISH for *C. glabrata* and *C. albicans* compared to routine identification methods were 100%. One patient isolate negative by FISH and presumptively positive for *C. albicans* by CHROMagar was identified as *C. dubliniensis*, a strain known to develop resistance to FLU. Tx of 7/15 patients (47%) with *C. glabrata* sepsis, was changed from FLU to CP within hours of reporting FISH results; for 2/10 patients (20%) with *C. albicans* fungaemia CP was switched to FLU after rapid reporting.

Conclusions: The PNA FISH accurately identifies *C. albicans* and *C. glabrata* directly from positive blood cultures within hours and can impact on the appropriate selection of the most effective antifungal Tx, thereby making it a clinically relevant diagnostic assay.

P1383 Evaluation of the new VITEK-2 *Neisseria haemophilus* card for the identification of fastidious organisms

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Objectives: The new VITEK 2 *Neisseria haemophilus* (NH) card (bioMérieux S.A) was evaluated for the identification of fastidious organisms. This card is based on colorimetric technology and results are interpreted with a computer-assisted algorithm that allows the identification of a total of 28 taxa.

Methods: A total of 73 well-characterised isolates of clinical samples, included in the VITEK 2 NH data-base, were evaluated: 7 *Neisseria* spp.; 13 *Haemophilus* spp.; 31 *Campylobacter* spp.; 7 *Moraxella catarrhalis*: and 15 isolates belonging to the HACEK group. 16S rRNA sequencing was used for the identification of the followings genera: *Aggregatibacter aphrophilus*, *Aggregatibacter actinomycetemcomitans*, *Capnocytophaga* spp., *Cardiobacterium hominis*, *Eikenella corrodens*, *H. parainfluenzae*, *Kingella kingae* and *Neisseria* spp. (except *N. meningitidis* and *N. gonorrhoeae* which were identified with API NH and home-made tests). Other genera such as *H. influenzae*, *M. catarrhalis* and *Campylobacter* spp. were identified with routine clinical laboratory tests.

Results: The VITEK 2 NH card correctly identified 70 (95.89%) isolates. Fifty-three (72.60%) of these were correctly identified without requiring further tests. An additional 17 (23.29%) isolates were identified with a low level of discrimination, 13 of which belonged to the genera *Campylobacter*. Further tests were required in these 17 cases to reach correct identification. Three (4.11%) isolates were misidentified (1 *C. coli*, 1 *C. jejuni*, 1 *N. meningitidis*).

Conclusions: This new NH card has an extended data-base and results are available within 6 hours. Both characteristics are advantageous in comparison with traditional methods when the isolates to be identified are previously correctly oriented. The new VITEK 2 NH card can thus be considered a good method for the identification of diverse groups of fastidious organisms

P1384 Evaluation of MALDI-TOF mass spectrometry for the identification of clinical yeast isolates

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Objectives: *Candida*, *Cryptococcus* and other yeasts are responsible for several systemic diseases such as fungaemia, meningoencephalitis, peritonitis and pneumonia. Conventional identification of these organisms is not always simple and reliable. Retesting and morphological examination can be necessary. On the other hand, due to different anti-fungal profiles, rapid presumptive species identification is essential. Matrix-Assisted Laser-Desorption/Ionisation Time-Of-Flight Mass Spectrometry (MALDI-TOF MS) is becoming a popular method for rapid identification of microorganisms. We compared the performance of this technique with conventional biochemical/physiological identification.

Methods: A total of 217 clinical isolates of yeasts and yeast-like organisms on Sabouraud agar were analysed by MALDI-TOF MS. They had been identified with chromogenic agar media (CANDI Select 4, BioRad, München, and/or API 32C (bioMérieux, Nürtingen) and additional tests if necessary. For sample preparation, a yeast colony was transferred into 300 μl of water, suspended and mixed with 900 μl of ethanol. After centrifugation the pellet was resuspended in 35% formic acid/50% acetonitrile and centrifuged. 1μl of the supernatant was used for measurement with alpha-cyano-4-hydroxy cinnamic acid matrix (Microflex LT, Bruker Daltonics, Germany). Bioinformatics was performed with the BioTyper 2.0 (Bruker Daltonics, Germany).

Results: Identical results were obtained with strains of *C. albicans* (n = 31), *C. glabrata* (n = 30), *C. tropicalis* (n= 31), five other *Candida* spp. (n= 39), and *Cryptococcus neoformans* (n = 3). Obviously due to gaps in the data base, MALDI-TOF MS provided no identification of *C. norvegensis* (n= 5), *Trichosporon* spp. (n= 4), and one strain each of *C. holmii*, *C. rugosa*, Geotrichum and *Pichia* sp. Introduction of a *C. norvegensis* database reference resulted in correct identification of the other strains. One strain of *C. dubliniensis* was identified on the genus level as *Candida*, best hit *C. albicans*. Introduction of a *C. dubliniensis* reference into the database resulted in correct identification. The method is robust, involving simple handling, low cost of consumables and high speed (10 min per test, 3 h for 96 samples).

Conclusion: MALDI-TOF MS represents a promising tool for rapid identification of the most important *Candida* species and related yeasts.

With completion of the data base, this technique may offer significant advantages over currently available methods.

P1385 Evaluation of the RIDA®QUICK Verotoxin/O157 for the detection of Shiga toxin and *Escherichia coli* O157 in stool enrichment cultures

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Objectives: Shiga toxins (Stx) are the major virulence factors of enterohaemorrhagic *Escherichia coli* (EHEC), which can cause diarrhoea and the life-threatening haemolytic uraemic syndrome (HUS), the major cause of acute renal failure in childhood. The serogroup O157 is associated with 70–90% of all EHEC infections. The prompt detection of the pathogen is mandatory to start the adequate therapy. We therefore evaluated the new RIDA®QUICK Verotoxin/O157, which detects Stx and the *E. coli* serogroup O157 within 16 hours.
Methods: To evaluate the RIDA®QUICK Verotoxin/O157 (R-Biopharm, Darmstadt, Germany), in total 161 samples were analysed. These included 51 EHEC reference strains which represent a broad spectrum of 38 different serotypes, all known Stx subtypes (except for Stx2g produced by animal strains only), and 6 Stx-low producers. Furthermore, 98 stools, 8 isolates from these stools and 4 animal isolates from our routine clinical microbiological laboratory and from the consulting laboratory were investigated for the presence of Stx and O157. The results were compared to genotypic detection of Stx encoding genes (stx) and of the sfpA gene that is specific for a subset of EHEC O157 by PCR. To verify Stx production the Vero cell cytotoxicity assay was applied in addition.
Results: Of the 161 samples, 145 samples (90.1%) were correctly diagnosed using the RIDA®QUICK Verotoxin/O157 regarding Stx production and/or serogroup O157. The serogroup O157 alone was correctly detected in 157 samples (97.5%) and Stx production was correctly determined in 147 samples (91.3%) in comparison to PCR detection and the cytotoxicity assay. Sensitivity, specificity, positive and negative predictive values for Stx/O157 detections were 84.3/91.7%, 98.7/98.5%, 98.6/91.7%, and 85.6/98.5%, respectively. False negative results of Stx detection were mainly due to low Stx production in isolates (n = 3), to low amounts of EHEC in the stool enrichment broth (5), and to the presence of rare Stx subtypes (Stx2d, Stx2e) as sole Stxs (2). The low EHEC concentration also explained 2 false negative O157 results. In two further samples, false positive O157 results were detected.
Conclusion: Overall, the new RIDA®QUICK Verotoxin/O157 is a highly specific method to determine the presence of Stx and the serogroup O157 in stool enrichment cultures within 16 hours. Ongoing studies investigate the test performance in comparison to Stx targeting ELISA.

P1386 Identification of staphylococci by using commercial kits STAPHYtest 24 and API Staph

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Objectives: Staphylococci represent a substantial part of the surface microflora of mammals and birds (skin, skin glands, mucous membranes) and some *Staphylococcus* species, mainly *S. aureus*, are found frequently as aetiological agents of a variety of human and animal infections. The species identification of staphylococci based on biotyping is still commonly performed in routine clinical laboratories. The aim of presented study was the comparison of species identification of staphylococci by using two commercial biochemical systems.
Methods: A total of 195 staphylococcal cultures were evaluated in this comparative study including 40 reference CCM strains and 155 clinical isolates obtained from 3 different microbiological laboratories. All cultures were identified by standard procedure (STAPHYtest 24 and API Staph) according to manufacturer's instruction. In case of discrepant identification results obtained by commercial kits the final identification was performed in the National Reference Laboratory for Staphylococci based on a large set of tests.
Results: STAPHYtest 24 kit successfully identified to the species level 160 cultures (82%) of tested, remaining 27 strains (14%) were identified to the genus level only and 8 strains (4%) stayed unidentified or misidentified. Applying of additional tests recommended by identification software (TNW v. 7.0) improved species identification to 94% when 6 strains (3%) were misidentified and next 6 strains (3%) stayed identified to genus level only. API Staph kit surprisingly identified only 103 cultures (53%) to the species level. Great number of strains, 71 of 195 studied (36%), was identified by API Staph just to genus level and 21 strains (11%) were not identified or misidentified. Applying of additional tests according to identification software (apiweb) improved correct species identification to 84%. Unfortunately this step increased false identifications to 30 cases and the high number of misidentified staphylococci represent a great problem of API Staph kit or identification software apiweb.
Conclusion: Our results proved usefulness of the new identification kit STAPHYtest 24 for reliable identification of staphylococci. In our view the STAPHYtest 24 is superior to API Staph as regards of identification efficacy as well as user friendly.

P1387 Differentiating *Pseudomonas aeruginosa* from common enteric bacteria by digital tinctorial properties

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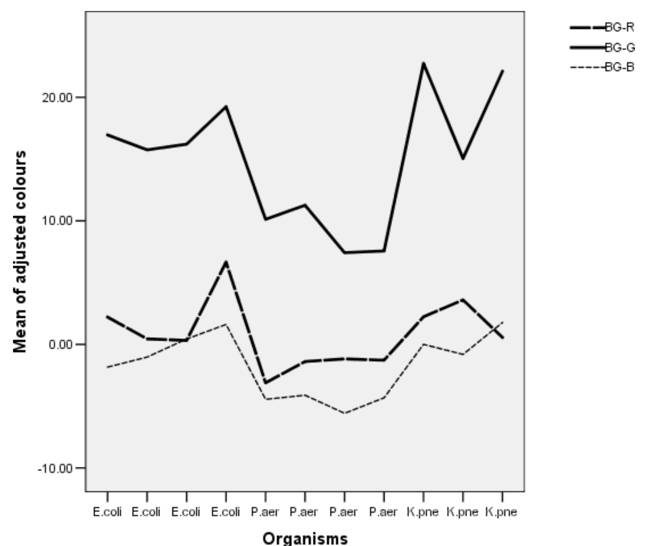
Objectives: *P. aeruginosa* is an important pathogen which demands a distinct empiric antimicrobial coverage. On Gram's staining, it has long been observed that *P. aeruginosa* stains less intensely by safranin O than common enteric bacteria. In this pilot study, we sought to determine staining characteristics which may aid clinicians in making an initial antimicrobial choice and minimising overusage of broad-spectrum coverage.
Methods: 4 isolates of *Escherichia coli*, 4 of *Pseudomonas aeruginosa* and 3 of *Klebsiella pneumoniae* were employed. On the Figure, the first isolate of each genus represent ATCC strains (25922, 27853, and 27736, respectively) followed by clinical isolates. After concurrent growth in culture media for over 24 hours, the isolates were transferred to 0.85% NaCl, adjusted to 0.5 McFarland and then smeared on slides by the same-size loops. A standard Gram's staining was performed. Microscopic pictures of the stained smear were digitally taken. 20 to 30 bacilli were then randomly selected from the pictures of each smear for analysis. Maximal digital colour value of each bacterium was recorded under Adobe Photoshop CS2 in the RGB mode with red (R), green (G) and blue (B) measured separately. The colour intensity ranges from 0 to 255. Intensity of the corresponding colour on the surrounding background of each bacterium was also recorded.



Results: 320 bacilli from the 11 isolates were selected – 120 for *E. coli*, 110 for *P. aeruginosa* and 90 for *K. pneumoniae*. Given a strong correlation between the colour intensity of the bacterium and that of its surrounding background and the variability of background intensities in different areas, the value of that of background minus that of the bacterium (BG-R, -G or -B) was used. The mean value of all the measured adjusted background/bacterium in each isolate was plotted (Figure). BG-R was 2.41, –1.79, 2.13; BG-G 17.04, 9.25, 19.97; BG-B –0.2, –4.53, 0.32 for *E. coli*, *P. aeruginosa* and *K. pneumoniae*, respectively. Intergroup mean difference between *E. coli*-*P. aeruginosa* and *P. aeruginosa*-*K. pneumoniae* were both significant ($p < 0.001$) for all colours. Mean difference between *E. coli*-*K. pneumoniae* was not significant for BG-R ($p = 0.916$), BG-B ($p = 0.422$), but significant for BG-G ($p < 0.001$).
Conclusion: Our study demonstrate a potential utility of digital tinctorial measurement as a diagnostic aid to guide appropriate empiric antimicrobials. Further analysis on more bacterial genera and from direct clinical specimens are needed.

P1388 Evaluation of the StrepB Select agar for the detection of group B streptococci from vaginal and recto-vaginal specimens

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Background Objective: Current guidelines for the prevention of group B streptococcal (GBS) perinatal disease are based on prenatal screening culture for recto-vaginal GBS colonisation. Use of selective and differential media as Granada type agar (GRA) or ChromID StreptoB agar (SBID) improves sensitivity and workload of these cultures. This study was conducted to evaluate performances of the new StrepB Select Agar (SBS) Bio-Rad, for the selective growth of blue-turquoise colonies of GBS.
Methods: 500 genital swabs collected from pregnant and non-pregnant women. Each swab was homogenised in 2 ml of sterile saline and 0.05 ml aliquots were inoculated onto SBS, modified GRA (Becton Dickinson), SBID (bioMerieux) and blood agar with colistin-nalidixic acid (CNA), primary cultures. The remaining suspension was added to a selective enrichment Lim broth. After overnight incubation, aliquots of Lim broth were inoculated onto SBS, GRA, SBID and CNA. SBS and SBID were incubated in air, GRA anaerobically and CNA in air + 7% CO_2, at 35°C, 24–48 h. Positive and negative control strains (GBS; *E. faecalis*) were cultured with each run. Specific identification of colonies suggestive of GBS (light blue to dark blue-turquoise on SBS, light pink to red on SBID, beta-hemolytic on CNA) was performed; orange colonies on GRA were identified as GBS.
Results: GBS were recovered from 147 swabs (29.4%): 111 from primary cultures and 139 after Lim enrichment, respectively from 103 and 134 on SBS, 90 and 123 on GRA, 93 and 124 on SBID, 76 and 113 on CNA. Overall sensitivities were 94.6% on SBS, 84.4% on GRA, 87.1% on SBID and 81.6% on CNA. Characteristic colonies of presumptive GBS were not always confirmed as GBS: 41 from primary cultures and 38 after Lim enrichment on SBS, 22 and 17 on SBID and 45 and 59 on CNA. Respectively the positive predictive values of presumptive GBS colonies were 71.5–77.9% (SBS), 80.9–87.9% (SBID) and 62.8–65.7% (CNA). At 48 h incubation, presumptive GBS were easily observed on SBS, GRA and SBID even in low numbers.
Conclusions: 1) The highest sensitivity was observed for SBS, followed by these on SBID and GRA. 2) Due to lack of specificity, characteristic colonies on SBS as on SBID or CNA must be isolated to confirm their identification. 3) Presumptive GBS were easily observed on SBS, GRA and SBID. 4) SBS as SBID are incubated in air and do not require CO_2 or anaerobic conditions. 5) SBS, a new useful agar to recommend for GBS prenatal screening culture.

P1389 Comparison of BACTEC Plus media and BacT/Alert FA media to detect bacteria in blood culture bottles containing peak therapeutic levels of antimicrobials

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Objectives: Antimicrobials given to patients before blood has been obtained can affect the detection of bacteria in subsequent blood cultures. Blood culture bottles with antimicrobial removal system have been developed and widely used. This study compared the ability of BACTEC Plus (Becton Dickinson, USA) bottles and BacT/Alert FA (bioMerieux Vitek, France) bottles to effectively remove antimicrobials.
Methods: BACTEC Plus and BacT/Alert FA bottles were spiked with 5mL human blood, peak therapeutic concentrations of antimicrobials (vancomycin, ampicillin, oxacillin, gentamicin, cefepime, ciprofloxacin, cefotaxime, cefoxitin, and ceftriaxone) and 7 type strains of *S. aureus* (meticillin susceptible and resistant), *S. pneumoniae*, *E. faecalis*, *E. coli*, *K. pneumoniae* and *P. aeruginosa* and a clinical isolate of meticillin resistant *S. aureus*. Three rounds of duplicate testing were completed per antimicrobials/strains combination and growth control without antimicrobials. After 5 days incubation, the time to detection (TTD) and recovery rates for bacteria compared with both automated blood culture systems.
Results: Overall, the BACTEC Plus and BacT/Alert FA recovered 76% (128/168) and 34% (57/168) of strains from test bottles, respectively and both media recovered 100% (48/48) of strains from growth control bottles. BACTEC Plus detected all of Gram-positive bacteria except *S. pneumoniae* with ampicillin (33%) and ceftriaxone (33%), but BacT/Alert FA detected 0–50% of Gram-positive bacteria except *E. faecalis* with vancomycin (100%) and meticillin-resistant *S. aureus* with oxacillin (100%). In presence of cefepime, cefotaxime, cefoxitin and ceftriaxone, BACTEC Plus detected 33–100% of Gram-negative bacteria, but BacT/Alert FA did not detect at all. In presence of ciprofloxacin, BacT/Alert FA detected 100% of *E. coli* and *K. pneumoniae* strains compared with 33% of those for BACTEC Plus. In case of gentamicin, both blood culture media were effective to detect all of Gram-negative bacteria. The overall TTD of BACTEC Plus was shorter than that of BacT/Alert FA.
Conclusion: In presence of β-lactam antimicrobials and vancomycin, BACTEC Plus media is more effective and faster detection of bacteria than BacT/Alert FA. In case of ciprofloxacin, BacT/Alert FA is superior to BACTEC Plus for detection of Gram-negative bacteria.

P1390 Evaluation of the VITEK® 2 NH card for identification of fastidious Gram-negative bacteria

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Objectives: Fastidious Gram-negative bacteria comprise a very heterogeneous group, some of them easily recognised by their morphological features or growth requirements, while for others the identification is both time consuming and difficult. The newly developed VITEK 2 NH card (BioMerrieux) covers 26 clinically important fastidious Gram-negative bacteria, including *Neisseria* and *Haemophilus*. In our study, we compared the VITEK 2 NH card with phenotypic and genotypic characterisation.
Methods: 100 clinical and reference strains, including 30 type strains, were examined. 75 of these represented 21 of the 26 taxa included in the VITEK 2 NH database. The remaining 25 strains mainly comprised species of *Neisseria* not included in the database plus Pasteurella spp. Performance was assessed to be correct when the strain was identified as the only choice with any level of confidence. Extensive phenotypic testing was performed according to classical methods and partial 16S rRNA gene sequencing (526 base pairs) was done with comparison of the edited sequences with deposited sequences in the “Bacteria” NCBI database.
Results: 45 of the 75 strains of taxa included in the database were identified correctly by the VITEK 2 NH card, including the *Neisseria*

species (including ProA-positive *N. gonorrhoeae*), the *Haemophilus* species, *Moraxella catarrhalis* and *Eikenella corrodens*.

Of the 22 strains incorrectly identified (n = 3) or identified with low discrimination (n = 37), the most problematic were the proA-negative *N. gonorrhoeae*, *Kingella* spp. and *Capnocytophaga* spp. Of the17 strains identified with low discrimination, meaning that two to three different taxa were given as equally possible, three strains were correctly identified with the use of some of the supplementary tests.

Of the 25 strains of taxa not included in the database, only 4 were correctly "unidentified", which means not recognised in the database. The 21 remaining strains were misidentified as belonging to some of the species included in the database.

Conclusion: The VITEK 2 is an easily used tool in the routine laboratory. The results in our study indicate that the VITEK 2 NH card will identify most of the commonly occurring species in the database correctly, except the proA-negative N.gonorrhoea. There are, however, identification problems when testing strains not included in the database, but deriving from the same genera as those in the database or from the same clinical infections, such as animal bite wounds.

Table 1. Identification of the 75 strains of 21 taxa included in the VITEK® 2 NH database and the 25 strains not included in the database

Conventional identification	Number of strains	Correct identification	Low discrimination	No identification	Incorrect identification
Included in the database					
Actinobacillus ureae	2*	1			1
Capnocytophaga canimorsus	6	1		2	3
Capnocytophaga gingivalis	1*			1	
Capnocytophaga ocharacea	1*	1			
Capnocytophaga sputigenea	1*			1	
Cardiobacterium hominis	2*	2			
Eikenella corrodens	3*	3			
Haemophilus (*Aggregatibacter*) *actinomycetemcomitans*	3*	2	1**		
Haemophilus (*Aggregatibacter*) *aphrophilus*	2*	2			
Haemophilus (*Aggregatibacter*) *paraphrophilus*	2*	2			
Haemophilus haemolyticus	1*				1
Haemophilus influenzae	3*	3			
Haemophilus parahaemolyticus	3*	1	1		1
Haemophilus parainfluenzae	3*	3			
Haemophilus (*Aggregatibacter*) *segnis*	3*		2***	1	
Kingella denitrificans	2*		1**		1
Kingella kingae	3*	1		2	
Moraxella (*Branhamella*) *catarrhalis*	3*	2	1**		
Neisseria cinerea	3*	2	1**		
Neisseria elongata	3*	3			
Neisseria gonorrhoeae	10*	5	2**		3
Neisseria lactamica	2*	2			
Neisseria meningitidis	10*	9	1*		
Neisseria sicca	2*	2			
Suttonella indologenes	1				1
Total	**75**	**47**	**10**	**7**	**11**
Not included in the database					
Actinobacillus hominis	3*		2		1
Moraxella non-liquefaciens	1*		1		
Moraxella osloensis	1*		1		
Neisseria animaloris (CDC EF-4a)	3*			2	1
Neisseria flavescens	1*		1		
Neisseria mucosa	2*				2
Neisseria pharyngis	1				1
Neisseria polysaccharea	1		1		
Neisseria weaveri	3		1		2
Neisseria zoodegmatis (CDC EF-4b)	3*				3
Pasteurella canis	1				1
Pasteurella dagmatis	1				1
Pasteurella multocida	3*			1	2
Pasteurella stomatis	1			1	
Total	**25**		**7**	**4********	**14**

*Type strain of species included among the strains.
**Correct identification included among suggested identifications.
***In 1 of 2 strains correct identification included among suggested identifications.
****Correctly unidentified strains.

P1391 Comparative evaluation of five *Aeromonas* phenotypic identification systems

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Accurate identification of *Aeromonas* strains involved in human infections with methods or systems usually encountered in clinical laboratories has never been extensively studied.

Objectives: to evaluate performances of 5 commercialised methods or systems in identifying *Aeromonas* strains in a clinical laboratory.

Methods: *Aeromonas* identification with 20E (A) and 32GN (B) strips (API, bioMérieux, France), GN card (C) (VITEK2, bioMérieux, France) NC47 (D) (Walk Away, USA) and NMIC/ID69 (E) (Phoenix, Becton Dikinson, USA) was carried out in a 5-centre evaluation. Testing was performed after control of each system. A total of 88 *Aeromonas* strains, of which 82 of clinical importance, was tested. Results were compared to partial rpoB gene sequence based identification used as gold standard. Species distributed as follows: *Aeromonas hydrophila* (n = 33), *A. veronii* (n = 30), *A. caviae* (n = 39), *A. jandei* (n = 3), *A. media* (n = 3), *A. allosaccharophila* (n = 3) and *A. bivalvium* (n = 3).

Results: Genus agreement was 94%, 100%, 99%, 94%, 98% with A, B, C, D, E respectively. Discrepancies dealt either with (i) erroneous genus identification (C, n = 3; D, n = 3), (ii) absence of result (E, n = 3) or (iii) no discrimination between two genus including *Aeromonas* (A, n = 3). Genus misidentification concerned *Vibrio* (n = 32) or *Francisella* (n = 3). Rates of identification to the species level was 33%, 22%, and 98%, with species agreement of 17%, 6% and 62%, with B, C, E systems respectively. The two remaining systems (A, D) never reached species level identification without additional tests being requested. Similarly, B and C systems requested additional tests for 80% of the strains. Additional tests, done as requested by the four systems enabled to increase rate of correct identification to the species level, although these tests may be variously easy-available in daily practice. The overall species agreement was 79%, 78%, 67%, 82% and 62% with A, B, C, D, E systems respectively.

Conclusion: Phenotypic methods or systems encountered in clinical laboratories show high performances in identifying *Aeromonas* strains to the genus level but low performances in identifying *Aeromonas* strains to the species level and should therefore be improved. *Aeromonas* identification should rely on more appropriate algorithms with available tests in daily practice, or on molecular methods.

P1392 Rapid identification of pneumococci and enterococci in blood culture bottles using Latex Agglutination

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Objective: To evaluate two commercial latex agglutination tests, for rapid identification of pneumococci and enterococci directly from positive blood culture bottles that showed the presence of Gram-positive diplococci by the Gram stain.

Methods: Period of study: From November 2005 to November 2007. Samples: All positive blood culture bottles where we visualised only Gram-positive diplococci by direct Gram stain. Agglutination test: 1 ml from the positive blood culture bottle was centrifuged at 2500 rpm for 10 min. To test Slidex pneumo-kit ® (bioMérieux), one drop of supernatant was mixed with one drop of latex reagent and to test the Streptococcal Grouping kit ® (Oxoid Diagnostic Reagents), 1 drop of supernatant was mixed with 1 drop of each latex group reagent (A, B, C, D, F, G). Boths reagents were mixed and rotated for 2 min. The results were evaluated comparing to the standardised identification methods from solid media.

Results: A total of 120 positive blood culture bottles were analysed with the following isolates: 87 *Streptococcus pneumoniae*, 16 *Enterococcus faecalis*, 14 *Enterococcus faecium* and 3 *Enterococcus* spp. – Firstly, the Slidex pneumo-kit ® to identify pneumococci showed a sensitivity of 91.5% and a 100% specificity, and the positive and negative predictive

values were 100% and 80%, respectively. Secondly, the Streptococcal Grouping kit ® evaluation for group D agglutination reagent to identify enterococci had a sensitivity of 75.8% and a specificity of 100%, and the positive and negative predictive values were 100% and 84.6%, respectively. In addition, we observed that 35 (79.5%) pneumococci showed positive agglutination with group C latex reagent. Summarising, both latex agglutination tests showed specificity values close to 100% and sensitivity values higher than 75% to identify pneumococci and enterococci.

Conclusion: The results of this study suggest that both reagents could be used to provide an earlier identification than standard methods of Gram-positive diplococci, and the combination of the two of them is useful to distinguish between pneumococci and enterococci. In adition both test are inexpensive and easy to perform in any laboratory.

P1393 PCR assay for detection of *Streptococcus dysgalactiae* ssp. *equisimilis*

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Objectives: *Streptococcus dysgalactiae* ssp. *equisimilis* belongs to Lancefield serogroup G, which forms heterogeneous group of beta-hemolytic streptococci. Presently, three taxonomic types of this serological group can be distinguished, as follows: minute colony formers from humans (*S. anginosus* group), and large colony formers from humans (*S. dysgalactiae* ssp. *equisimilis*) and animals (*S. canis*). In humans, they may colonise pharynx, skin, gastrointestinal and female genital tract. In recent years, they have been reported with increasing frequency as a cause of variety of human infections, such as endocarditis, arthritis, pharyngitis and sepsis. The differentiation of streptococci traditionally relies on the evaluation of growth, serological and biochemical properties. However, developments in genetic technology have resulted in new methods that can be used for identification of bacteria. The aim of the present work was the development of new PCR assay for the rapid and reliable identification of *S. dysgalactiae* ssp. *equisimilis*.

Methods: Based on the sequence analysis of the 16S-23S rDNA intergenic spacer region of eight *S. dysgalactiae* ssp. *equisimilis* strains of human origin species-specific primers were designed using the MEGA program, version 3.1. Subsequently, a PCR with these primers was optimalised and was used to identify *S. dysgalactiae* ssp. *equisimilis* strains isolated from humans.

Results: The oligonucleotide primers designed according to the species-specific parts of the 16S-23S rDNA intergenic spacer region yielded an amplicon with a size of 205 bp for all *S. dysgalactiae* ssp. *equisimilis* strains investigated. *S. canis*, also belonging to Lancefield's serogroup G, and all the other control strains of various species and serogroups were negative throughout. The limit of detection of *S. dysgalactiae* ssp. *equisimilis* was determined to be 1.4 ng DNA.

Conclusion: PCR method presented in this study allowed a rapid and reliable identification of *S. dysgalactiae* ssp. *equisimilis* and might help to improve the diagnosis of this bacterial species in human infections. Although this species doesn't belong among common streptococcal species, its importance should not be underestimated, because it might be the cause of various infections, including serious, live-threatening states. This work was supported by MSM 0021627502.

P1394 Evaluation of clinical and laboratory parameters for establishing the diagnosis of bacteraemia caused by coagulase-negative staphylococci

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Objectives: Coagulase-negative staphylococci (CNS) are both an important cause of nosocomial blood-stream infections and the most common contaminants of blood cultures. In this study we evaluated clinical significance of CNS bacteraemia by analysing clinical parameters of CNS bacteraemia, slime production, incubation times for blood cultures to become positive, number of positive blood culture bottles and antimicrobial susceptibility of isolated CNS.

Methods: Between June 2006-June 2007, 710 blood cultures (BactAlert 3D, bioMereux) positive for CNS from 458 patients were collected. Diagnostic criteria of Rabdom Medical Center were used for determination of true bacteraemia. CNS were identified by growth in sheep blood agar, Gram staining, catalase and coagulase tests. Further identification and antimicrobial susceptibility analysis were done by using VITEK2 (bioMerieux) automated system. Slime production was determined by Congo Red agar test. Statistical analyses were performed using the statistical software SPSS for Windows 10.0.1.

Results: According to Rabdom Medical Center diagnostic criteria 80 (17.5%) of the CNSs isolated from blood cultures were causes of true bacteraemia and 378 (82.5%) were contaminants. *Staphylococcus epidermidis* (48.5%) was the most frequently isolated species associated with true bacteraemia. Also it was the most frequently isolated contaminant (84.2%). Slime production was significantly higher in isolates that caused true bacteraemia than contaminants ($p < 0.05$). 85% of CNS that were isolated from true bacteraemia were resistant to meticillin whereas % 60.6 of contaminant strains were resistant ($p < 0.005$). CNS isolates from true bacteraemia were significantly more resistant to meticillin, gentamicin, erythromycin, clindamycin ($p < 0.05$). There was no significant relation between number of positive blood culture bottles and true bacteraemia ($p > 0.05$). The cut-off value for incubation time for blood cultures to become positive was determined 19.4 hours according to ROC analysis. Incubation time was significantly shorter for blood cultures of true bacteraemia cases than contaminants ($p < 0.05$).

Conclusion: In this study, we concluded that no single method has a high accuracy to diagnose a true CNS bacteraemia. Both clinical and laboratory parameters should be evaluated. Species identification of CNS, incubation time, slime production and determination of antimicrobial susceptibility can be all useful tools for determination of clinical significance of CNS isolates.

P1395 Evaluation and implementation of a chromogenic agar medium for the detection of *Salmonella* in stool in routine laboratory diagnostics

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Objectives: Each year our laboratory processes more than 5,000 stool samples from patients with gastro-enteritis for culture of *Salmonella* species. On conventional agar media recognition of *Salmonella* is generally based on lactose fermentation and H2S production. Specificity is low and lactose-fermenting salmonellae may go undetected. We evaluated which chromogenic *Salmonella* agar medium would improve the sensitivity and specificity of our *Salmonella* stool culture protocol and thereby decrease workload and costs.

Methods: In the first phase of the study, we tested BBL CHROMagar *Salmonella* (BBL; Becton Dickinson, The Netherlands), SM ID2 (SM; bioMérieux, France) and Oxoid *Salmonella* Chromogenic Medium (OX; Oxoid, United Kingdom) with 34 *Salmonella* strains and 19 non-*Salmonella* strains. Secondly, we prospectively analysed the two deemed most appropriate media with stool samples submitted from general practice patients with gastro-enteritis. The chromogenic media were processed alongside the conventional media Salmonella–Shigella agar (SS), Xylose-Lysine-Desoxycholate agar (XLD) and Hektoen-Enteric agar (HE) according to the current stool culture protocol. This included subculturing after broth enrichment and presumptive identification of colonies suspected of being *Salmonella* using conventional biochemical tests.

Results: With stock isolates, sensitivities of BBL, SM and OX were 100%, 94% and 85% respectively after both 24 h and 48 h of incubation. Specificities were 84%, 90% and 74% after 24 h and 68%, 79% and 53% after 48 h respectively. Based on this, we continued the study with BBL and SM. Of 1339 stool samples, 32 (2.4%) were *Salmonella* positive on at least one medium. After direct plating and enrichment sensitivities for SS, XLD, HE, BBL and SM were 72%, 97%, 84%, 88% and 78%,

specificities were 79%, 80%, 81%, 96% and 96% respectively. On SM more overgrowth of faecal flora was found compared to BBL. XLD and BBL together yielded a sensitivity of 100%. The higher specificities of the chromogenic media reduced the required amount of presumptive identification considerably.

Conclusion: Of the chromogenic media tested, we found BBL the most appropriate substitute for conventional *Salmonella* media. We expect a combination with XLD to give the best results for *Salmonella* and to provide optimal isolation of *Shigella*. Adaptation of the culture protocol to this effect will result in a substantial decrease of costs of *Salmonella* diagnosis in our laboratory.

P1396 Benchmarking of microbiology laboratories

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Objectives: A multi-centre evaluation was performed in order to compare activities and procedures in microbiology laboratories of 5 hospitals with similar activities in Belgium. This evaluation was considered a first step towards standardisation of microbiology procedures.

Methods: Five microbiology laboratories were provided with a questionnaire including parameters measuring hospital size, staffing, organisation and activities (2004). Consecutively, labs were visited and the provided data were discussed. Two well defined procedures (MRSA screening and blood culture) were observed and compared. Participation was voluntary and based on a network of collaborating microbiologists.

Results: The number of hospital beds in the 5 centres ranged from 460 to 939 (median 822, day care included). All hospitals had a medical/surgical intensive care unit (range 20–34 beds). Although three centres were multi-site hospitals, all of them had their microbiology activities centralised in one laboratory. Activities resorting under microbiology were bacteriology (n = 3), molecular biology (n = 3), fertility (n = 3), serology (n = 3) and virology (n = 3). The total number of aerobic cultures performed (2004) ranged from 26040 to 59218 (median 44285) or 0.17 to 0.28 culture/hospital day, and 0.6 to 1 culture/hospitalisation period. Work load per laboratory technician ranged from 3219 to 5401 aerobic cultures/full time equivalent/year. The proportion of aerobic cultures with AST (antimicrobial susceptibility testing) performed, ranged from 17 to 32%. The number of blood cultures in each centre was 0.03–0.04 samplings/hospital day, and 0.10–0.15 samplings/hospitalisation period. All centres used automated blood culture systems, 1 centre used automation for AST reading, 2 centres for identification & AST. For MRSA screening all centres used a chromogenic agar, three of them had a procedure of enrichment. The number of screening samples/year ranged from 1885 to 9597 (median 6670), with 7 to 20% new positive patients/total number of patients screened.

Fast results and close consultation with the clinicians concerning AB therapy were key points in all centres.

Conclusions: Numbers and procedures were compared in five microbiology labs of Belgian hospitals with similar activities. Some data were remarkably comparable while other parameters differed widely. Labour-intensity of different tests should be considered when comparing numbers as a reflection of productivity rates.

P1397 Comparative evaluation of three selective media for primary isolation of *Helicobacter pylori* from gastric biopsies under routine conditions

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Objective: Culture of *Helicobacter pylori* from gastric biopsies allows diagnostic of infection, susceptibility testing to antimicrobials and study of virulence factors. The purpose of this study was to compare, under routine conditions, three selective media for the primary isolation of *H. pylori*.

Methods: From October 3rd to November 21st 2007, the study included gastric biopsies (1 per patient) from consecutive endoscopies submitted for routine culture of *H. pylori* to our laboratory. Media tested included two commercial: PYL (bioMérieux, France) and Helicobacter agar (Becton Dickinson, USA) and a home made medium (BHA: Brugmann Helicobacter Agar). Specimens were immediately treated or frozen at -70°C until the day of culture. Biopsies were ground in 1 ml sterile distilled water and 250μl of the final suspension was inoculated on each medium by circular streaking with a bent pipette. Plates were incubated for 10 days at 37°C under microaerophilic conditions. The growth of *H. pylori* was checked after 3 days then daily thereafter. If necessary, the identification of *H. pylori* was confirmed by the presence of Gram-negative curved bacilli and a positive test for urease, oxidase and catalase production. Additionally, number of colonies, days of positivity and presence of contamination were recorded. Chi Square test was used to compare the overall recovery rate on the different media. Days of appearance of colonies and average counts were compared by Friedman test with Dunn post test.

Results: Biopsies taken from 295 patients (aged from 1 to 96, mean 36 years) were plated in parallel on the media. By combining the results of the three media, the isolation rate of *H. pylori* was 23% (68 of 295 biopsies). Of the 68 samples, respectively 60, 61 and 62 were positive with BHA, PYL and Helicobacter agar, given an isolation rate of 88.2%, 89.7% and 91.2%. Average bacterial counts and day of positivity were respectively 20.286, 26.239 and 25.366 colonies/ml and 5.3, 4.6 and 4.3 days. Significance difference was observed only between BHA and PYL according to the number of colonies ($p = 0.011$). Extending incubation to more than 5 days showed contamination in PYL agar (10/295: *Aspergillus* sp.) and Helicobacter agar (7/295: 5 *Bacillus* sp., 1 Micrococcus, 1 *Pseudomonas* sp.). There wasn't any contamination in BHA.

Conclusion: The three media are acceptable for primary *H. pylori* isolation. BHA offers the advantage to be the most specific medium.

P1398 *Helicobacter heilmanii* (*Gastrospirilum hominis*) in gastric biopsies of humans – A fifteen-year analysis

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Objectives: The "non-*pylori*" representatives of the genus *Helicobacter* have recently been widely discussed. They have been described in connection with human enteral infections, sepsis, and infectious vasculitis. Special attendance was devoted to H. heilmanii. This Gram-negative stained spiral shaped rod with a characteristic morphology is in humans associated with chronic gastritis, ulcerous stomach disease and it is suspected to be connected with the risk of lymphoma formation. Immune response is similar to that observed in *H. pylori*, though it is less intensive.

Diagnostics of *Helicobacter heilmanii* is only based on microscopic evidence, although utilisation of molecular-biological methods is possible, yet not applicable in the routine practice. Until recently *Helicobacter heilmanii* has been considered a non-cultivable bacteria; though in 1999 some publication on a successful cultivation evidence from human material were published, cultivation in a routine laboratory remains rather problematic. We present our experience on this topic.

Material and Methods: The authors analysed incidence of *Helicobacter heilmanii* in 8179 bioptic samples of stomach mucosa acquired from two gastroenterological out-patient departments in the period between 1993 to 2007.

Material delivered to the laboratory in a transport medium was divided for urease production evaluation, cultivation a microscopic examination (Gram and Giemsa stain and starting 1998 also stained by Acridine orange).

Results: 2739 (33.49%) samples were positive on *Helicobacter pylori*, 31 (0.37%) were microscopically positive on *Helicobacter heilmanii*. In the five cases we detected in the same sample both *Helicobacter* spp. together.

Patients with H. heilmanii have less severe course of the disease and reacted well to the therapy. Irrelevant were data concerning animal contact.
Conclusion: We confirmed the published incidence of *Helicobacter heilmanii* (0.1–3%) in bioptic samples. Growing knowledge on its pathogenic activity are in agreement with the increased interest in the diagnostic laboratories.

P1399 A reassessment of in the diagnosis of brucellosis in Iran: ELISA or PCR?

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Objectives: Brucellosis is a worldwide infectious disease that poses an important public health problem especially in Iran. The development of a definite diagnostic test for brucellosis has been actively pursued. In the present study, the value of ELISA as an alternative method for the diagnosis of brucellosis has been investigated and compared with peripheral blood PCR assay.
Methods: A total of 37 consecutive patients with definite diagnosis of brucellosis (confirmed by blood culture and standard agglutination test) in Infectious Diseases Department of Imam Hospital, Medical Sciences/University of Tehran were prospectively evaluated. Moreover, blood samples from 78 controls (34 healthy controls and 44 non-brucellosis febrile patients) were included in the study. The peripheral blood PCR assay and ELISA IgG test were performed in patients and controls. In control group we calculated the mean of IgG levels plus 2 standard deviation (SD) as a cutoff value for ELISA IgG. The PCR target sequence of 223-bp present on a gene encoding a 31-kDa *Brucella abortus* antigen was selected for amplification.
Results: The blood culture was positive in 21 patients. The SATs were 1:160 and 1:320 in 35 (94.5%) and 28 (75.6%) patients, respectively. The positive results of PCR were observed in 15 (40.5%) patients and none of the controls. In patients and controls the mean levels of ELISA IgG were 171.33 ± 72.27 IU/ml and 19.78 ± 41.71 IU/ml, respectively. The cutoff point (mean plus 2 SD) was 103.2 IU/ml. The ELISA IgG had positive results in 33 (89.1%) patients and 4 (5.1%) controls. In 4 patients with negative results of ELISA IgG, serum level of IgM was measured by ELISA test. We found that the serum IgM level was more than 200 IU/ml in these 4 patients. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were reported 40.5%, 100%, 100%, 78% for PCR assay and 89.1%, 94.8%, 89.1%, 94.8% for ELISA test.
Conclusion: The results of the present study suggest that the ELISA test is the most sensitive method for the diagnosis of brucellosis and with the calculation of cutoff point it would be a reliable test. PCR assay is promising but standardisation of method is lacking and more investigations should be performed for better accuracy.

P1400 Distribution of *Nocardia* species in clinical specimens in Greece

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Objectives: The aim of this study was to assess the species distribution of a large number of *Nocardia* isolates in Greece and to propose a rapid identification test that may be helpful to identify the species most commonly encountered in clinical material.
Methods: A total of 100 *Nocardia* spp. isolates, recovered from clinically significant specimens, were identified using API-ZYM, according to the procedures of manufacturer and a molecular method based on hsp65 gene. The API-ZYM system, including 19 enzymes (alkaline phosphatase, esterase lipase, leucine arylamidase, acid phosphatase, phosphohydrolase, alpha-glucosidase, beta-glucosidase. valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, alpha-galactosidase, beta-glucoronidase, N-acetyl-beta-glucosaminidase, alpha-mannosidase, alpha-fucosidase, beta-galactosidase, ONPG) determined the enzyme profile of *Nocardia* species. On the other hand, DNA extracted from *Nocardia* species was amplified using as target the hsp65 gene. PCR products were then, purified and sequenced, while, identification to the species level was done after comparison with all sequences available at the BLAST Genome.
Results: Results obtained by API-ZYM system failed to discriminate the isolates to the species level. On the other hand, molecular method identified twenty isolates as *N. flavorosea*, thirty-four as *N. cyriacigeorgica*, twenty-six as *N. nova*, two as *N. veterana*, twenty-four as *N. farcinica*, two as *N. abscessus*, and two as *N. asteroides*.
Conclusions: Genus-specific hsp65 gene sequencing can be a rapid and reliable adjunct in the diagnosis of *Nocardia* to the genus and species level.

P1401 Differentiation of *Prevotella intermedia* and *Prevotella nigrescens* by MALDI-TOF-MS

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Objectives: The *Prevotella intermedia* group bacteria (black-pigmented Gram-negative anaerobic rods commonly found in dental biofilm) includes two phenotypically indistinguishable species *P. intermedia* and *P. nigrescens*. In order to facilitate identification of these two species from periodontal disease, bacterial identification by matrix-assisted laser desorption/ionisation mass spectrometry (MALDI-TOF-MS) was evaluated with the present study.
Methods: A total of 37 strains (*Prevotella intermedia* DSM 20706, *Prevotella nigrescens* DSM 13386 and 35 recent clinical isolates from 33 patients with aggressive periodontitis) were used. The clinical strains were very well identified as *P. intermedia/nigrescens* by rapid ID 32 A system (bioMerieux, Lyon, France) and species differentiation was done using sequence analysis of 16S rARN gene. In addition MALDI-TOF-MS was performed. All mass spectra were acquired using an Autoflex II (Bruker Daltonics, Bremen, Germany) operated under the Flexcontrol software ver.2.4 (Bruker Daltonics). Automated peak extraction was done with Flexanalysis ver. 2.4 (Bruker Daltonics).
Results: Although the MS spectra of the two species are visually rather similar the mathematical similarity analysis performed with MatLab 7.3 (The MathWorks Inc., Natick, U.S.A.) could well differentiate them. The spectra of the *P. intermedia* strains identified with MALDI clustered together into two subtrees which are significantly different from *P. nigrescens*. The latter fell also into two different subtrees. The quality of clustering was characterised by calculating an inconsistency coefficient (Mathworks: /Matlab Reference Manual v2007a/, Statistical toolbox). The reproducibility of the method was demonstrated by the similarity of spectra belonging to same species. These results are of clinical importance since there are several studies which associate *P. intermedia* with periodontal disease and *P. nigrescens* with a healthy periodontium. However, conflicting data were also published. The availability of a straightforward method to identify the two species might be helpful to clarify this problem.
Conclusion: This study proves that MALDI-TOF-MS is a new accurate method capable of separating these two species that cannot be readily identified by biochemical analysis. With databases for various groups of bacteria expanding, MALDI-TOF-MS combined with non-linear statistics becomes an attractive system even for routine identification of clinical isolates.

P1402 High performance and acceptability of self-colllected anal swabs for diagnosis of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in men and women

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Objectives: Identification of sexually transmitted infections (STI) is hampered by the limited compliance to undergo anal STI testing.

Therefore, alternative non-invasive diagnostic procedures are needed. This study asesesss usability of self-collected anal swabs (SAS) in diagnosing anal *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) among men who have sex with men (MSM) and women. The study is performed in two regions of the Netherlands with different level of urbanisation and risk group composition.

Methods: In 2006–2007, MSM and women who attended the Amsterdam and South Limburg STI clinics and reported receptive anal sex were asked to fill in a questionnaire and provide SAS in addition to standard provider collected anal swab (PAS). Swabs were tested for CT and NG using nucleic acid amplification tests (NAAT; Roche, Becton Dickinson). In Amsterdam PAS are also tested for NG by culture. Positive NG samples were confirmed by NAAT. Sensitivity (Se), specificity (Sp) and kappa (>0.8 good test agreement) of SAS compared to PAS were calculated. Positive and negative predictive value (PPV, NPV) was calculated for SAS.

Chlamydia trachomatis		Provider swab		self-swab		PPV	NPV	κ
		+	–	Sens.	Spec.			
	+	135	14					
MSM self-swab	–	10	1244	88%	99%	91%	99%	0.88
	+	62	6					
Women self-swab	–	4	643	94%	99%	91%	99%	0.92

Neisseria gonorrhoeae		Provider swab		self-swab		PPV	NPV	κ
		+	–	Sens.	Spec.			
	+	56	13					
MSM self-swab	–	8	848	88%	98%	81%	99%	0.83
	+	7	1					
Women self-swab	–	1	500	88%	99%	86%	99%	0.87

Results: Of the participants (1411 MSM, 715 women) CT prevalence was 11% (MSM) and 9% (women) and NG prevalence was 7% and 1%. CT prevalence was slightly higher in Amsterdam (highly urbanised). In 2% of MSM and 1% of women there was discrepancy between SAS and PAS test results. For NG this was 2% and 4%. SAS performance for CT and NG diagnosis was good in MSM and women (see tables) and was comparable for both study regions.

Of all participants, 68% filled in questions on SAS acceptability. Of respondents, 78% of MSM and 85% of women would prefer SAS in stead of PAS or had no preference (percentages highest in South Limburg). Only 6% of MSM and 5% of women would not use SAS again and 3% would not visit the STI clinic again when SAS was the standard test. Ten percent of MSM and 14% of women found SAS uncomfortable (percentage lowest in South Limburg); 3% of MSM and 4% of women found test instructions unclear.

Conclusions: Considering that substantial part of the population has anal sex (i.e. 10–20% of all men and women attending STI clinics) and anal STI is frequently present, anal screening should be essential part of an STI consultation. Self-collection of anal specimens is feasible, valid and acceptable for MSM and women in STI clinics. It may be a valid method for anal screening (especially for CT) in other settings as well.

P1403 Comparison of standard test for bacterial endotoxin detection with a rapid slide method

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Objectives: Testing surveillance and environmental samples for endotoxin content with the standard test procedure (The LAL endochrome test from Charles River Laboratories) performed at Rigshospitalet is often laborious. Furthermore the extend of pipetting work may in the long run cause arthritis like conditions in the fingers of the technicians working with the LAL method.

Charles River Laboratories has developed a revolutionary FDA-licensed endotoxin detection system, the Endosafe PTS, which is a portable and handheld rapid point-of-use test system comprising a simple one-button operation. Both the LAL test and the Endosafe PTS method are based on a kinetic chromogenic methodology.

This study compares the LAL method with the new PTS method.

Methods: A total of 18 tests are performed with the LAL method and the PTS method.

The first step is to dilute an *E. coli* control standard Endotoxin manually, thereby creating a standard dilution row. The 18 tests are separated into 3 standard rows each comprising 6 concentrations (0; 0.05; 0.12; 0.25; 0.50; 1.00 EU/ml). From this point the 18 dilutions are subjected to the LAL procedure first (lasting about 1.5 h) and to the PTS method in the meantime. The PTS method involves an application of 25 ml of sample to 4 wells on a slide, which is then inserted into the PTS apparatus and the endotoxin measurement is completed within a maximum of 15 minutes. Thus the PTS system requires a minimum of hands-on time.

The measured concentrations of the dilutions in both the LAL and the PTS method are based on optical densities.

Results: The results are viewed in table 1, which shows the endotoxin concentration measurements from the LAL method and the PTS method.

Conclusion: When making a graph, based on the values in table 1, it is clearly seen that the tendency with the LAL method is that the values are unevenly increasing until the 0.50 point and then the curve decreases. However the measurements in the PTS method are following a straight line increasing approximately two-fold, which agrees with the manually produced dilution rows. Thus a conclusion must be that the standard method produces uncertain results, most likely caused by an error in the reader device, and that the PTS system conducts more reliable, all though systematically low, measurements.

Table 1: Endotoxin measurements from LAL method and from PTS method

Dilution, EU/ml	Row 1		Row 2		Row 3	
	LAL	PTS	LAL	PTS	LAL	PTS
0	<min	<0.01	–0.191	<0.01	<min	<0.01
0.05	0.076	0.022	–0.005	0.018	–0.172	0.016
0.12	0.276	0.039	0.475	0.048	0.063	0.045
0.25	0.587	0.085	0.377	0.084	0.544	0.084
0.50	>max	0.16	0.794	0.22	0.823	0.176
1.00	>max	0.436	0.688	0.429	>max	0.423

P1404 Soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) for distinguishing bacterial (Empyema) from non-bacterial pleural effusion

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Objectives: Exudative effusion is a common clinical condition among hospitalised patients which might be secondary to infection, malignancy, lymphatic abnormalities, and non-infectious inflammation. Biochemical parameters used nowadays to distinguish bacterial from non-bacterial pleural effusion before the results of pleural fluid cultures are available, have wide zones of overlap between the different causes of exudative effusion. A rapid microbiologic tool is the Gram stain but its sensitivity is low, approximately 50%. Triggering receptor expressed on myeloid cells (TREM)-1 is a recently identified molecule that is involved in monocytic activation and in the inflammatory response. Studies have shown that TREM-1 is up regulated by microbial products in the plasma, bronchoalveolar lavage fluid, and cerebrospinal fluid of patients with sepsis, pneumonia and bacterial meningitis, respectively.

Two recent studies showed that the soluble TREM-1 (sTREM-1) levels were significantly higher in infectious pleural effusions compare to

pleural effusion of other aetiology. However, in those studies sTREM-1 levels in definite empyema versus exudative pleural effusion due to other inflammatory processes, such as Dressler's syndrome were not evaluated. We aimed to evaluate whether sTREM-1 in exudative pleural effusion can predict a bacterial infection (pleural empyema).
Methods: Patients of all ages who present with pleural effusion were identified through laboratory records. In addition to routine biochemical markers, differential white blood cells, cytology, Gram stain and pleural fluid culture, pleural fluid sTREM-1 was measured by ELISA using a commercial kit (R&D Systems Minneapolis, USA). Empyema was defined as aspiration of pus, a positive Gram stain or culture results, pH < 7.2, a glucose level <40 mg/dl, and an LDH > 1000 IU/L.
Results: Thirty-eight patients were included in the study. Seven patients had empyema, 3 – simple parapnomonic effusion, 8 – transudate, 5 – Dressler's syndrome, 8 – malignancy, 1 – connective tissue disease, and 6 patients had undetermined effusion. Mean levels of sTREM-1 were significantly higher in empyema (614 ± 184 pg/ml) compared with the other effusions (61 ± 42 pg/ml, p =0.03).
Conclusions: Our findings suggest that sTREM-1 is up-regulated in the pleural effusion of patients with empyema and can potentially assist clinicians in early early differentiation of bacterial.

Regional spread of MRSA and MSSA

P1405 Reduced transmission of animal-related ST398 meticillin-resistant *Staphylococcus aureus* in Dutch hospitals

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Objectives: To determine the transmission rate of animal-related ST398 MRSA and other MRSA isolates within Dutch hospitals.
Methods: Data were collected in 51 Dutch hospitals from July 2006 to January 2007. An index patient was defined as a carrier of MRSA (not necessarily infected) that was treated in a hospital without transmission precautions. As part of the Dutch MRSA policy all possible contact patients and healthcare workers (HCW) are screened for MRSA after identification of an index patient.
Results: During 306 months of observation, there were 80 index patients: 24 ST398 and 56 with other MRSA genotypes. Other genotypes included typical hospital-associated genotypes, but not USA300. In 80 clinical post-exposure surveys 2134 patients and 5758 HCWs were screened for MRSA. ST398 MRSA carriage was found in 3 HCWs (0.3% of all screened HCWs) as compared to 62 secondary cases with other MRSA genotypes (0.6% of screened HCWs; 1.7% of screened contact patients). No transmission of ST398 MRSA to screened contact patients (n = 383) was documented. Index patients carrying ST398 MRSA had been hospitalised shorter before isolation measures were taken than carriers of other MRSA genotypes (median respectively 1.5 and 4 days; p = 0.04); the number of contacts screened per day of exposure, however, did not differ between both groups (p = 0.75). The risk of transmission of ST398 MRSA per day of exposure was 25% of the risk of spread of the other MRSA genotypes (95% CI = 0.08 to 0.78; p = 0.01).
Conclusion: During 306 hospital-months the risk of transmission of pig-related ST398 MRSA was lower than that of other MRSA genotypes. Not a single documented case of patient-to-patient spread was found.

P1406 MRSA surveillance in the Netherlands shows a different geographical dissemination of pandemic clones and the high diversity of the Dutch MRSA population

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Objectives: MRSA prevalence is much higher in other European countries than in the Netherlands. Little is known about cross-border migration of MRSA. Although not fully unambiguous, PFGE and spa typing allow comparison of internationally distributed strains. Here we focus on PFGE types en spa types which are part of major international clonal clusters, i.e. CC5, CC8, CC45 and CC80
Methods: Over the first 11 month period of 2007 2730 unique MRSA isolates were sent to RIVM for subtyping. All strains were characterised by PFGE and spa typing. Isolates were labeled according to the postal code of the laboratories which submitted the strains. Maps were created to show the geographical distibution of strains belonging to major international clones. Minimum Spanning Trees (MSTs) were generated to depict the proportion of the major clones among the diverse Dutch MRSA isolates. Simpson's diversity indices were calculated for PFGE and spa typing.
Results: 495 strains were characterised as PFGE and spa types which belong to international clones. Strains belonging to CC8, CC22 and CC80 were randomly distributed in the Netherlands. The strains which belonged to CC45 were predominantly found in the south of the province of Zuid-Holland and in Zeeland, Noord-Brabant and Limburg especially in the border regions. This might indicate introduction of these strains from cross-border regions with high MRSA prevalence. MST's of both PFGE and spa types showed the high diversity of the Dutch MRSA population which was also reflected by the calculated diversity indices.
Conclusions: A strain known as(Dutch) PFGE type 113, spa type t038 or closely related t740 and belonging to CC45 present along southern border region in the Netherlands has not dispersed to other parts of the country.
Other major clones are present in all dense populated areas in the Netherlands.
The Dutch MRSA population displays a high degree of diversity and further reseach on the spread of specific clones is needed.

P1407 Molecular epidemiology of PFGE non-typeable meticillin-resistant *Staphylococcus aureus* in the Netherlands

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Objectives: Recently, pigs were identified as a possible new reservoir for community-acquired meticillin-resistant *Staphylococcus aureus* (CA-MRSA). These pig-related MRSA strains can easily be identified by pulsed-field gel electrophoresis (PFGE). In PFGE, no banding pattern is obtained, due to methylation of the SmaI site, and was from then on referred to as non-typeable (NT)-MRSA. From 2003 to 2007 the number of NT-MRSA was increasing rapidly and pig-to-human transmission had occurred.
The present study gives an overview of the NT-MRSA in the Netherlands in 2007, the characteristics of the NT-MRSA and the clonal structure of these strains. The data will show the importance of this relatively new CA-MRSA strain.
Methods: In the Netherlands, the National Institute of Public Health and the Environment (RIVM) serves as the national reference centre for surveillance of MRSA. All first MRSA isolates, one per patient, are sent to the RIVM for PFGE typing. From this MRSA database all NT-MRSA isolates of 2007 were used for further characterisation.
Molecular typing included staphylococcal protein A (spa) typing, staphylococcal cassette chromosome mec (SCCmec) typing, and multilocus sequence typing (MLST).
Results: Up till November 2007, a total of 535 MRSA isolates were non-typeable by PFGE. Spa typing revealed 24 different spa types. Most of the spa types were closely related, indicating a clonal complex. The two most prevalent spa types were t011 and t108. All NT-MRSA belonged to SCCmec type IV or V and were ST398.
Conclusion: In 2007 approximately 30% of all MRSA isolates were non-typeable. This new CA-MRSA of zoonotic origin appears to spread rapidly, not only in the Netherlands but also in Europe. In the Netherlands MRSA guidelines are changed due to this new strain.

P1408 Molecular characterisation of meticillin-sensitive *Staphylococcus aureus* bloodstream isolates in a Dutch university hospital between 1999 and 2006

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Objectives: It is suggested that meticillin-resistant *Staphylococcus aureus* (MRSA) originated through the transfer of the mobile resistant determinant staphylococcal cassette chromosome mec (SCCmec) into meticillin-susceptible *S. aureus* (MSSA), and that the genetic background determines the stability of MRSA. To support this hypothesis, MSSA bloodstream isolates (BSI) in our hospital were characterised and the genetic background of the MSSA clones compared to endemic MRSA clones.

Methods: A random selection of MSSA BSI (n = 380), isolated between 1999 and 2006 in the University Hospital Maastricht (azM), were characterised with spa typing and the algorithm based upon repeat pattern (BURP). Furthermore, the prevalence of the virulence factors collagen adhesion (CNA), Panton-Valentine leukocidin (PVL) and toxic shock syndrome toxin 1 (TSST-1) was investigated.

Results: Up to 50% of the MSSA isolates had a genetic background common to the endemic MRSA clones, e.g. clonal complex (CC) 1, CC5. CC8, CC22, CC30, and CC45. Furthermore, several CCs not related to the endemic MRSA clones, such as CC7 and CC15 (12% and 7% of the isolates respectively), were observed. CNA, PVL and TSST-1 was observed in 42%, 2%, and 15% of the isolates respectively. CNA was associated with CC1, CC12, CC22, CC30, CC45, and CC121, while TSST-1 was associated with CC30.

Conclusion:

1. Half of the MSSA isolates had a genetic background common to endemic MRSA clones.
2. Several MSSA lineages, such as CC7 and CC15, were prevalent in our hospital.
3. The prevalence of MSSA clones with genetic backgrounds both common and uncommon to MRSA clones, supports the observation that SCCmec is more stable in certain *S. aureus* genetic backgrounds.
4. Although endemic MRSA clones could originated through the transfer of SCCmec into MSSA, it seems more likely that MRSA clones have been imported from abroad, rather than originated in The Netherlands.

P1409 Diversity of the virulence and genomic backgrounds in clinical isolates of *Staphylococcus aureus* collected in a Spanish hospital 1992 to 2006

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Staphylococcus aureus is an important human pathogen. The major virulence factors involved in pathogenesis are controlled by the agr (accessory gene regulator) quorum-sensing system. In this work, we studied the relationship between virulence (V) and genetic backgrounds of 30 non-invasive and 31 invasive isolates recovered in the Monte Naranco Hospital (Asturias, Spain) over 1992 to 2006. All isolates were typed by SmaI macrorestriction-PFGE followed by UPGMA-cluster analysis, and the V-gene profile was determined by PCR with primers specific for toxins and agr genes. In meticillin resistant isolates, the type of SSCmec was also identified.

The 61 isolates displayed 42 PFGE profiles, falling into 8 clusters and 11 branches, at S=0.6. One major cluster (15 SmaI-profiles including 25 invasive isolates) and 7 minor clusters (6 with non-invasive, and one with invasive isolates) were revealed. i) All isolates were positive for the hemolysin hla and hld genes and the leukotoxin lukED gene. The prevalent profile was hla-hlb-hld-hlg-hlg-variant (92%) with lukPV (26%). ii) 92% of the isolates were also positive for exfoliative toxins genes (et). The prevalent was etb, which appeared either alone (41%) or in combination with eta (20%), etd (26%) or both (5%). iii) 29.5% was positive for tst, present only in invasive isolates and frequently associated to agr group III. iv) 98% was positive for classic enterotoxin genes (se), all of them carrying the enterotoxin gene cluster (egc), in addition to other se-genes. Statistically significant differences between invasive and non-invasive isolates were: sea (45% vs. 93%), seb (6.5% vs. 47%), sec (87% vs. 50%), see (0% vs. 13%), and seu (55% vs. 0%). Regarding the agr type, invasive isolates carried agr group III (35%), while 20% of non-invasive isolates were positive for both agr group I and II (and possibly carried an agr-hybrid). Among the meticillin-resistant isolates, 71.4% within the invasive group contained SSCmec IVc, while the non-invasive group carried SSCmec I, SSCmec III or recombinant derivatives.

In summary, *S. aureus* causing invasive and non-invasive diseases in a Spanish Hospital were distributed in different genomic types. Despite of important differences in the genetic background, isolates of both groups are in possession of a very high number of common virulence factors. However, genes encoding potent virulence factors (lukPV, eta and tst) were more frequently found in the invasive isolates.

P1410 Emergence of a multi-resistant genotype of meticillin-resistant *Staphylococcus aureus* in a university hospital in Barcelona

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Objectives: Meticillin-resistant *S.aureus* (MRSA) has been endemic in Hospital Universitari de Bellvitge (HUB) since 1990. The majority of strains isolated during the 1990–95 period belonged to the multiresistant Iberian clone (ST-247; SCCmec-I). However, isolates of Clonal Complex 125 (ST-125; SCCmec-IV), close related to the Paediatric clone, were dominant from 1996 to 2003. MRSA isolates resistant to erythromycin, clindamycin, gentamicin, tobramycin, ciprofloxacin and showing decreased susceptibility to rifampin (DS-Rif) were first detected in 2004. These DS-Rif-MRSA isolates increased steadily from 20% (57/282) in 2004 to 45% (116/256) in 2006. The aims of this study were: the molecular characterisation of these multiresistant strains and the analysis of the mutations involved in Rif resistance.

Methods: A selection of non-duplicate DS-Rif-MRSA clinical isolates (n = 308) collected from HUB between 2004 and 2006 were studied. Susceptibility to Rif was performed by the disk-diffusion method and MICs were studied by E-test. The Rif resistance-associated mutations in the gene rpoB encoding the RNA polymerase beta-subunit were analysed. Genotyping was performed by PFGE, spa typing, SCCmec and MLST (ST).

Results: 104 strains had intermediate resistance to Rif by disk-diffusion method and 4 were resistant. In the Rif-intermediate group, 13 isolates were susceptible by E-test (MICs 0.75–1 mg/L) according to CLSI criteria. All 108 isolates presented the mutation His481/Asn conferring low-level Rif resistance. Four isolates with MIC >32 mg/L had an additional mutation Glc468/Lys, Ala477/Thr, or Ile527/Leu, responsible of high level Rif resistance. The 108 isolates belonged to a single PFGE pattern, carried SCCmec type I, ST-228 and a spa type t041.

Conclusion: A multiresistant MRSA clone (ST-228; SCCmec-I), related to the Southern Germany clone, emerged in our hospital in 2004 and currently represents half of the MRSA in our institution. This clone has been reported in other hospitals in Spain since 1996, as well as in other European countries, however none of these reports had informed of the decreased susceptibility to Rif. The majority of strains isolated in our institution showed a single mutation in the rpoB gene, conferring low-level Rif resistance. One tenth of these isolates were misclassified as Rif susceptible by CLSI criteria.

P1411 Molecular epidemiology of meticillin-resistant *Staphylococcus aureus* in Santander, Spain

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Objective: To analyse the molecular epidemiology and susceptibility of meticillin-resistant *Staphylococcus aureus* (MRSA) in our area (Santander, Spain) in the period January 04-December 06.

Methods: In the study period, 1431 MRSA were isolated from clinical samples of 525 patients. After identification and susceptibility testing, pulsed-field gel electrophoresis (PFGE) and staphylococcal chromosomal cassette mec (SCCmec) typing were performed on 755 isolates, corresponding to the first isolate from each patient (including isolates from different wards or with different resistance phenotype from the same patient). Multilocus sequence typing (MLST) was performed on representative isolates from each PFGE type. Finally, 572 isolates (one isolate per patient, PFGE pattern and resistance phenotype) were selected for data evaluation. We compared data taking into account if patients were or were not hospitalised at the moment of MRSA isolation.
Results: We have identified the following clones: 74.6% of ST125-MRSA-IV (45.0% for admitted patients and 55.0% for non-admitted patients), 15.2% of ST5-MRSA-IV (46.0% and 54.0%), 3.7% of ST8-MRSA-IV (33.3% and 66.7%), 3.5% of ST228-MRSA-I (70.0% and 30.0%) and other 3.0% of minority clones including: ST22-MRSA-IV, ST247-MRSA-I, ST398-MRSA-IV, ST125-MRSA-V, ST36-MRSA-II and two new ones with non-described mutations. We found a proportional distribution of the main clones over the three year period. All isolates were susceptible to vancomycin. The percentages of susceptibility for the main clones to ciprofloxacin, erythromycin, clindamycin and gentamicin were as follows: ST125-MRSA-IV (0.2%; 17.1%; 73.2% and 60.1%), ST5-MRSA-IV (0%; 71.3%; 90.8% and 73.6%), ST8-MRSA-IV (57.1%; 85.7%; 100% and 100%) and ST228-MRSA-I (0%; 5%; 10% and 0%).
Conclusions: There are 4 main clones in our area that have been maintained over the study period. These clones were observed among both hospitalised and not hospitalised patients.

P1412 Molecular characterisation of Panton-Valentine leukocidin positive *Staphylococcus aureus* invasive isolates in Spain

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Objectives: Panton-Valentine leukocidin (PVL) is a *Staphylococcus aureus* exotoxin associated with skin and soft-tissue infections and severe necrotising pneumonia. The aim of this study was to determine the prevalence of PVL+ *S. aureus* isolates from blood cultures in selected Spanish hospitals and to characterise these isolates by molecular methods.
Methods: Twenty-one Spanish hospitals participated in a multicentre study (September 2006 to February 2007) collecting the first five successive meticillin-susceptible *S. aureus* (MSSA) and meticillin-resistant *S. aureus* (MRSA) isolated from patients with invasive infections. The antimicrobial susceptibility was determined by the disk-difussion and microdilution methods according to the CLSI guidelines. PVL detection was performed by PCR following the method of Lina. Several multiplex PCR were used for to determine the SCCmec types and the agr-types. All isolates were genotyped by PFGE after digestion of chromosomal DNA with SmaI. MLST and spa-typing were also performed.
Results: A total of 203 unduplicated *S. aureus* isolates (90 MRSA and 113 MSSA) were collected. Of these, 42 isolates (20.7%) were PVL+ (only one MRSA). The unique PVL+ MRSA isolate was resistant to oxacillin only, SCCmec type IV, agr group I, spa-type t032 (singleton), ST8 and showed a PFGE profile closely related to the community-acquired MRSA (type A). The molecular markers in the PVL+ MSSA isolates were as follows: agr-type I, 36.6%; II, 56.1%; III, 4.9% and IV, 2.4%; MLST-types ST5, 29.3%; ST15, 19.5%; ST25, 12.2%; ST8, 9.8%; ST121, 4.9%; 2.4% each type ST1, ST12, ST26, ST125, ST188 and ST789; 4 strains, 9.8% had new types; spa-types t002, 17.1%; t067, 9.8%; 7.3% each type t008 and t084; 4.9% each type t078, t148 and t346; 16 strains, 38.4% with several spa-types; in 2 strains the spa-type could not be determined. PFGE analysis showed 8 clones including 87.8% of PVL+ MSSA, and 5 sporadic strains.
Conclusions: The prevalence of PVL+ in MSSA invasive strains was very high in Spain (37.1%), but not in MRSA (1.1%). A half of these PVL+ MSSA isolates were MLST types ST5 or ST15 and a quarter were t002 or t067 spa-types; these MLST- and spa-types were also present in Spanish PVL– MRSA. Surveillance studies are necessary for the control of PVL+ *S. aureus* invasive strains.

P1413 Community-acquired meticillin-resistant *Staphylococcus aureus* in a reference childrens' hospital in Spain

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Objectives: Community-acquired meticillin-resistant *Staphylococcus aureus* (CA-MRSA) infection is an emerging health problem in children and adults. The objectives were (1) to assess if paediatric CA-MRSA infections are increasing in Barcelona, Spain, (2) to characterise the clinical manifestations associated with CA-MRSA infections comparing with hospital-acquired infections MRSA (HA-MRSA), (3) To identify the presence of risk factors in children with CA-MRSA infections.
Methods: A retrospective study included all children patients (<18 years) attended at the Sant Joan de Déu Hospital during the period 2001–2006, with positive culture for MRSA. MRSA were identified by standard microbiological methods. MIC determinations were performed for oxacillin and other antimicrobials by using Microscan Walkaway instrument. HA-MRSA was defined as one isolated from a specimen obtained beyond 72 hours of admission. Isolated rates were calculated as incidence infection per 100,000 populations.
Results: During the study period we identified 85 isolated for MRSA in 77 patients (52% boys, 48% girls) with mean age 6.5 years (SD 6.1). Of the total patients included, 66.2% (51) was CA-MRSA and 33.8% (26) was HA-MRSA. CA-MRSA infection increased from 13 infected patients during 2001–2003 to 38 infected patients during 2004–2006. Comparing the two-study period, the rates of CA-MRSA increased from 2.4 to 6.1 episodes/100,000 children <18 years (an increase of 155%, 95% CI 36%-379%; $p = 0.002$). Abscess predominated in CA-MRSA patients (82.3%) and surgery wound infection in children with hospital-acquired infections (27%), ($p < 0.001$). Among CA-MRSA patients, only 19.6% presented risk factors vs. 84.6% in HCA-MRSA group ($p < 0.001$). Community acquired-pneumonia (4.0%) was presented in patients with chronic neurological diseases, whereas hospital acquired-pneumonia (23%) affected in patients with prematurity and immunosuppression.
Conclusion: The appearance and spread of CA-MRSA represent a new challenge in paediatric patients and have important clinical implications for therapy of infections caused by *S. aureus*. A high level of clinical suspicion and development of rapid diagnostic methods are matters to be solved in the future.

P1414 Molecular characterisation of resistance to mupirocin in meticillin-resistant *Staphylococcus aureus* isolates in a tertiary hospital in Spain

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Objectives: Mupirocin(Mup) resistance appears either by a mutation, leading to low level resistance to mupirocin (LMupR), or as a result of the gene (ileS2) located in a plasmid and expressing high level resistance to mupirocin (HMupR). The aim of study was to determine the prevalence of MupR in meticillin resistant *S. aureus* (MRSA) in our institution, and to compare the epidemiologic and molecular characteristics between the MupR and MupS MRSA isolates.
Methods: We conducted a retrospective study between Jan and Dec 2006. All isolates were screened for resistance to Mup on Mueller-Hinton agar with a 5 μg disk. MupR isolates underwent MIC analysis by the Etest strip method. PCR detection of the genes mecA and ileS2 was performed. MupR and MupS isolates were typed by Pulsed Field Gel Electrophoresis (PFGE) following DNA extraction and digestion with SmaI, multilocus sequence typing (MLST) and (SCC)mec typing. The clinical charts of all patients were revised and clinical and epidemiological data were collected.
Results: Of the 207 MRSA isolates available for testing 23 (11.1%) were resistant to mupirocin. We found 18 isolates (8.7%) with HMupR

(MIC > 256 mg/L) and 5 (2.4%) with LMupR (MIC = 8–256 mg/L). In all isolates with HMupR the ileS2gene was detected by PCR. No isolate with LMupR had this gene. Gentamycin was more frequent (66.7%vs 13.2% $p < 0.001$) in the group of the MupR isolates. The rest of antibiotics had similar resistance patterns in both of the detected groups. Regarding epidemiological and clinical characteristics between MupR and MupS MRSA isolates we did not observe any significant difference. Molecular analysis of the MupR isolates revealed that 15 (83.3%) belonged to ST125-IV, 2 isolates belonged to ST228-I and only one belonged to ST45-IV. In the MupS isolates, the ST distribution was similar to the MupR group, except for the ST8-IV presented only in the MupS isolates. All the LMupR isolates belonged to only one clone ST228-I.
Conclusions: Although the rate of HMupR isolates has not significally progressed since our last study in 2001, it is important to monitorise the high resistance to mupirocin, because the transmission of MupR-MRSA strains and the horizontal plasmid transfer can contribute to increase the resistance to mupirocin in our institution.

P1415 Meticillin-resistant *Staphylococcus aureus* with exofiative toxin A in England and Wales during 2006–2007

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Objectives: Exfoliative toxin A (ETA) is associated with staphylococcal scalded skin syndrome (SSSS) and bullous impetigo. Symptoms include skin lesions, widespread erythema and exfoliation. Traditionally ETA has been associated with 'skin associated' phage group II MSSA isolates. More recently community strains of MRSA positive for ETA have been reported in Switzerland and Japan. We sought to characterise CA-MRSA with ETA identified at the England and Wales Staphylococcal Reference Laboratory (SRL).
Methods: Putative CA-MRSA isolates referred to SRL in 2006–7 were tested by PCR for the presence of toxin genes, including ETA. ETA positive isolates were characterised by spa typing and SmaI PFGE. PCRs detected: mecA and the SCCmec cassette type, ccr type and agr allotype. MICs of a range of antibiotics were determined. Patient demographic and clinical data were retained for analysis.
Results: Eighteen ETA-MRSA were detected during 2006–7. Eight patients were male, 8 were female. Ages ranged from 0–90 years (mode = 0 y, median = 30 y), three patients were >60 y, 6 were <10 y, two isolates had no patient information. Two patients were community-based, four isolates occurred in two centres, the remainder occurred sporadically. Eight isolates were from skin swabs, four from wound swabs, one each from; a screening swab, sputum, abscess, eye infection and an undisclosed source. Seven isolates were CC88-SCCmecIV and related by spa typing; two of these were multi-resistant and one encoded PVL in addition to ETA. A further eight isolates were CC5-SCCmecIV, were related by spa typing and encoded various staphylococcal enterotoxins in addition to ETA; one was multi-resistant. Two isolates were CC45-SCCmecIV, one of which was multi-resistant. The remaining isolate was CC121-SCCmecIV and positive for ETB in addition to ETA.
Conclusion: The data show multiple lineages of ETA-positive CA-MRSA have been identified in the UK. Their ability to cause skin disease, notably in young individuals and evidence of association with PVL demand increased vigilance. This early alert will assist in the monitoring of these new strain types as they occur.

P1416 Characterisation of community and hospital-associated *Staphylococcus aureus* isolates in Southampton

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Objectives: Meticillin-sensitive *S.aureus* (MSSA) can acquire the meticillin resistance determinant mecA, in a mobile genetic element known as the staphylococcal cassette chromosome-mec (SCCmec), giving rise to meticillin resistant *S.aureus* (MRSA). Community-associated (CA)-MRSA strains appear to contain the smaller, more mobile versions of the cassette such as SCCmec IV and V, and some strains may produce Panton-Valentine leukocidin (PVL). We used molecular typing to characterise hospital (HA) and community-associated (CA) *S.aureus* isolates circulating in Southampton, UK and to investigate the spread of PVL and SCCmec types IV and V.
Methods: We examined 71 CA and HA-MRSA/MSSA isolates (Southampton General Hospital in-patients and patients recently admitted or community healthcentre attendees). Isolates were typed by pulsed-field gel electrophoresis (PFGE), sequence types (STs) identified by multi-locus sequence typing and PCR used to determine SCCmec type and detect lukFS for PVL.
Results: Fifteen PVL positive isolates were identified, ten of which were MRSA that contained SCCmec IV. Seven of these were ST 30. Of the fifteen PVL positive strains, seven were community-associated, four of which were MRSA. Predominant STs were 22 and 30. Amongst ST 22 (n = 38), 12 were confirmed as MRSA, all were PVL negative, and 15 were hospital-associated. Amongst ST 30 (n = 3), 7 were confirmed as MRSA, all of which were PVL positive, and 5 of which were hospital-associated. The predominant PFGE type was EMRSA-15 and related subtypes. For both ST 22 and 30, there was no correlation between site of acquisition and meticillin-resistance.
Conclusions: Our studies reveal the presence of the mobile mecA-determinant SCCmec IV in CA- and HA-MRSA isolates some of which are also PVL positive. SCCmec V was present in two HA-MRSA isolates. The additional presence of PVL positive MSSA in community and hospital-derived isolates suggests a risk of gene transfer from SCCmec IV MRSA to PVL positive MSSA.

P1417 Dissemination of a new CA-MRSA strain in the UK

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Objective: Recently we detected a new CA-MRSA strain (ST97-MRSA-SCCmecV) which can harbour two copies of the arcA locus. The additional arcA locus is associated with the Arginine Catabolic Mobile Element (ACME) suggested as a putative virulence or fitness factor in USA300–0114. We sought to determine the dissemination of this new ST97-MRSA-SCCmecV strain in England and Wales.
Methods: All putative CA-MRSA isolates referred to the national staphylococcus reference laboratory in 2005–7 were tested by PCR for the presence of toxin genes. Isolates positive for staphylococcal enterotoxins D and J only were tested for spa type, SCCmec type, ccr type and ACME-arcA. MICs of a range of antibiotics were determined and SmaI PFGE performed. Patient demographic and clinical data were collated.
Results: Fourteen SCCmecV MRSA with ACME-arcA and staphylococcal enterotoxins D and J were detected. The isolates occurred in three geographically distinct centres in patients with an age range 61–93 years (median 81 years, mode 89 years). Data were available for seven of the fourteen isolates and indicated all seven were associated with skin and soft tissue infection. The fourteen isolates were closely related by PFGE (⩽2 band changes) and by spa typing (t359 and t267). All were agr 1, harboured SCCmecV and ccrC. The isolates were resistant to β-lactams, and had variable resistance to erythromycin, ciprofloxacin, clindamycin and/or trimethoprim.
Conclusions: This new strain type was identified in elderly patients in multiple diverse locations in the UK, and was strongly associated with sporadic skin and soft tissue infections. This strain type can be multi-resistant and encodes a factor possibly associated with skin survival or transmissibility and requires increased vigilance.

P1418 EARRS-SEQNET surveillance of *S. aureus* associated with bacteraemia in the UK during 2006

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Objectives: Systematic data regarding the molecular epidemiology of *S. aureus* in the UK are limited. However, studies from the late 1990s

indicated that EMRSA-15 and 16 dominated the bacteraemia-associated-MRSA landscape in the UK. Through the EARRS-SEQNET initiative we aimed to assess the molecular epidemiology of MRSA and MSSA associated with bacteraemia in the UK in 2006.
Methods: Up to ten successive MSSA and MRSA isolates from different patients were collected from 12 participating laboratories in England, Wales and Northern Ireland from September 2006. *S. aureus* were characterised by phage typing, PCR for mecA, lukSF-PV and spa, and sequencing of spa amplicons. MICs of a wide range of antibiotics were determined. Patient demographic and clinical data were retained for analysis.
Results: 111 *S. aureus* blood isolates were included for study. For patients with MRSA, ages ranged from 25 to 92 years (mode=79y, median = 30y); 66% were male; 29% were community-based; and 24% died within 14 days of specimen date. For patients with MSSA, ages ranged from 0 to 95 years (mode=0y, median = 30y); 53% were male; 58% were community-based; and 8% died within 14 days of specimen date. EMRSA-15 accounted for 94% of the 53 MRSA isolates, 90% of MRSA isolates tested resistant to ciprofloxacin; none tested resistant to glycopeptides or linezolid. Amongst these, t032 was the most frequent spa type (34/53 MRSA), various other spa types along with phage typing indicated a further 16 isolates to be E-15. One EMRSA-16 (t018) was identified; the two remaining MRSA were t127. Amongst the 58 MSSA, over 40 different spa types were identified. Two MSSA isolates with unrelated spa types (t2663 & t2864) encoded PVL.
Conclusion: Differences in key demographic data were identified between MSSA and MRSA cases. EMRSA-15 (CC22) remains the predominant MRSA associated with bacteraemia in the UK, the MSSA are more diverse genotypically, including representatives of 5 major clonal complexes (CC1, 5, 8, 22 and 30).

P1419 Absence of MRSA in clinical mastitis samples recovered from dairy cattle across England and Wales

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Objective: Meticillin resistant *Staphylococcus aureus* (MRSA) is a major cause of healthcare associated infection and is under investigation as a zoonotic pathogen. The current study was conducted to determine the prevalence of MRSA in dairy cattle across England and Wales. In addition, the presence of toxin genes and susceptibility to other clinically important antimicrobials were investigated.
Method: *S. aureus* isolates (n = 300) were recovered from mastitic milk samples, and screened by a duplex PCR for the species-specific nucA gene and the meticillin resistance gene, mecA. Plate dilution and disc diffusion susceptibility testing was performed against 10 clinically important antimicrobials. 250 strains were selected for PFGE-SmaI characterisation on the basis of antibiogram and geographical data. Of these, 100 isolates with distinct PFGE and/or antibiograms were examined by multiplex PCR for the presence of 14 genes encoding staphylococcal toxins (namely, enterotoxin A-E and G-J, exfoliative toxins A, B and D, toxic shock syndrome toxin-1 and Panton Valentine Leukocidin toxin).
Results: All 900 isolates were nucA positive and mecA negative. Antimicrobial sensitivity data showed 412 isolates (46%) were resistant to $\leqslant 1$ antibiotics. The most common resistance was against penicillin (96.1%). Resistance to remaining antimicrobials was as follows; ciprofloxacin (10.7%), oxacillin (6.1%), erythromycin (4.9%), amoxicillin/clavulanate (2.2%), cefoxitin (2.7%), tetracycline (4.4%) and gentamicin (1%). PFGE analysis demonstrated 47% identity amongst the 250 strains. Toxin characterisation revealed 43% of isolates possess at least one toxin. Staphylococcal enterotoxins and TSST-1 were detected as follows; 13.2% (sec), 1.4% (sed), 27.9% (seg), 27.9% (sei), 1.4% (sej) and 14.7% (tst). All isolates were negative for PVL and exfoliative genes (eta, etb and etd).
Conclusion: The findings from this study suggest dairy cattle are not reservoirs of MRSA and associated PVL toxin across England and Wales. Dairy cattle could be a potential reservoir of TSST-1 and enterotoxins but not exfoliative toxins. Resistance to penicillin was found to be high, this has been shown in many similar animal studies and relates to the widespread usage of penicillin. There is a need for continued surveillance of other farmed livestock to understand origins and epidemiology of MRSA in the UK.

P1420 Community-associated meticillin-resistant *Staphylococcus aureus* (CA-MRSA) in southeastern Austria: distribution to spa-types and first occurrence of USA300

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Objective: Infections due to community associated *Staphylococcus aureus* (CA-MRSA) are a recent worldwide phenomenon. To estimate the prevalence of CA-MRSA in Austria, determination of the Panton-valentine leukocidin (PVL) and spa-typing of the CA-MRSA was performed, followed by a synopsis of the clinical features in the affected patients.
Methods: A total of 2484 non-duplicated *S. aureus* strains, isolated in 2005 at the Institute of Hygiene, Medical University Graz, Austria, were investigated. The isolates were characterised by routine laboratory standard methods and the resistance pattern according to the CLSI guidelines was determined. Additionally polymerase chain reaction tests for *S. aureus*-specific genes, the mecA gene and the Panton-Valentine leukocidin (PVL) gene were performed. Spa Typing was used for characterisation of the CA-MRSA strains and the detected spa types were compared with spa databases.
Results: 84 (3.4%) out of the 2484 *S. aureus* isolates were identified as MRSA, while 13 (16.0%) out of these 84 MRSA strains were identified as CA-MRSA, with a positive reaction in the PCR for the PVL-gene. Patients with CA-MRSA had no risk factors for colonisation by healthcare associated MRSA (HA-MRSA), such as recent hospitalisation and/or contact with healthcare workers. All PVL-positive CA-MRSA strains were non-multiresistant – in addition to the resistance to oxacillin these CA-MRSA strains exhibited resistance only to the following antibiotics: Resistance to fusidic acid, tetracycline and erythromycin was found in 46% of the strains each, ciprofloxacin in 31% and gentamicin in 8% of the strains.
Spa typing showed 5 different Spa types: t004 (5 strains/ CA-MRSA widely disseminated in Europe), t008 (4 strains/ Northern German MRSA, USA300), t622 (2 strains), t001 (1 strain/ Southern German MRSA) and t355 (1 strain).
Clinical features of the CA-MRSA patients were superficial to deep soft tissue abscess formation with single up to multiple abscesses. After surgical intervention and antibiotic therapy all patients recovered without sequelae. Pneumonia could not be observed in any case.
Conclusions: In 2005 16% of MRSA primary-isolates were identified as CA-MRSA, which indicates that CA-MRSA spread more and more into the community in patients in Southeast Austria. Distribution to spa types shows a tendency of the CA-MRSA to well described European CA-MRSA strains, and for the first time the occurrence of USA300 in Southeast Austria is could be observed.

P1421 Close links between nosocomial MRSA and carriage MSSA isolates from the same location in Switzerland

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Staphylococcus aureus is a notorious nosocomial pathogen but it also harmlessly colonises a large fraction of healthy human population. Studies which simultaneously survey both carriage strains from healthy carriers and hospital-associated MRSA are rare. Such studies are, however, necessary to better understand evolution of this pathogen, and in particular MRSA. Resistance to meticillin in MRSA is conferred by the mecA gene situated on a mobile genetic element called SCCmec. It has been assumed that acquisitions of SCCmec by MSSA are relatively rare.

The nasal swabs were collected from 405 newly employed hospital personnel during their first medical check-up at the tertiary care hospital in Lausanne between October 2005 and July 2006. During 2005 and 2006, 1386 MRSA isolates from hospitalised patients of the same region were analysed. All isolates were typed by DLST, a newly developed, high resolution method based on sequencing of partial repeats sequences of clfB and spa. In addition, carriage MSSA isolates were typed with MLST.
130 out of 405 (32%) hospital employees were *S. aureus* carriers. Typing with DLST allowed distinguishing 115 unique DLST types and only 37 MLST types showing that DLST has superior resolution in comparison to MLST. Genetic diversity among carriage strains was extremely high, with majority of carriers colonised with a unique strain. In contrast, among hospital MRSA isolates nearly 50% were of a single DLST type. To our surprise, all locally predominant clones of MRSA were very closely related (single locus DLST type) to the MSSA counterparts encountered in carriers. What is more, the carriage isolates closely related to epidemic MRSA clones were also the most abundant ones.
It appears that the same genotypes make the most efficient colonisers as well as the most successful epidemic clones. This strongly indicates that epidemic behaviour of these strains is inherent to their genetic background independently of acquisition of resistance determinants.

P1422 Regional variation and temporal trends of Meticillin-resistant *Staphylococcus aureus* using the Sentinel Surveillance of Antibiotic Resistance in Switzerland (SEARCH)

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Objectives: The epidemiology of Meticillin-resistant *Staphylococcus aureus* (MRSA) shows large geographic variations. Recently, a comprehensive national antibiotic resistance database covering in- and outpatients became available in Switzerland.
Methods: We used a laboratory-based active surveillance system (SEARCH, www.search.ifik.unibe.ch) of in- and outpatients in Switzerland with *S. aureus* isolated from any source to analyse regional and temporal trends in the epidemiology of MRSA.
Results: The proportion of meticillin-resistance in *S. aureus* was highest for long-term facilities (26.2%), followed by general hospital wards (7.4%) and Intensive Care Units (5.4%) and lowest in outpatients (3.5%) ($p < 0.05$ for each comparison). MRSA was more frequent in males than females (RR 1.19; $p < 0.001$) and increased with older age (p for trend <0.001). Meticillin-resistance tended to increase since 2004 from 6.2% to 6.6% in 2007 (p for trend 0.06) with significant increases over time in the proportion of MRSA within most age groups and for females (from 5.3% in 2004 to 6.2% in 2007; p for trend <0.001) but not for males. The proportion of MRSA among *S. aureus* from general practitioners and long-term facilities tended to increase since 2004, whereas no change was evident for hospital isolates. Marked geographic variations were observed with highest proportions of MRSA in the South (17.1%), followed by the West of Switzerland (10.4%) and lowest proportions in Central (3.9%) and Eastern Switzerland (3.8%). While there was a significant increase in the East (3.2% in 2004, 4.0% in 2007; p for trend 0.04), elsewhere prevalence remained stable (Central) or showed a non-significant increase (West: 8.9% in 2004, 10.7% in 2007) or decrease (South: 18.1% in 2004, 15.9% in 2007). Cotrimoxazole (3.8%) and tetracycline resistance (7.9%) in MRSA remained low in 2007, while 43%, 66% and 74% were resistant against clindamycin, erythromycin and ciprofloxacin, respectively. Resistance rates of MRSA against clindamycin and ciprofloxacin decreased significantly since 2004. Temporal resistance trends for individual antibiotics varied markedly across regions. There was a trend towards an increase of non-multidrug resistant MRSA since 2004 (p for trend 0.09). The risk of MRSA was slightly higher during the cold season than in warmer months (RR 1.05; p 0.04).
Conclusion: MRSA prevalence remains low in Switzerland but both prevalence and temporal trends differ widely across regions.

P1423 Molecular characterisation of community- and hospital-acquired meticillin-resistant *Staphylococcus aureus*

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Objectives: The aim of this work was to investigate the presence of the Panton-Valentine leukocidin (PVL) gene in Community-Acquired Meticillin-Resistant *Staphylococcus aureus* (CA-MRSA) collected from outpatients in the San Paolo Hospital of Milan from April 2006 to July 2007. Those isolates were also investigated for their SCCmec mobile generic element, coding for meticillin resistance. For comparative purposes we included in this study a matching number of Hospital-Acquired Meticillin-Resistant *Staphylococcus aureus* (HA-MRSA).
Methods: We collected twenty six CA-MRSA isolates derived from skin and soft tissue specimens as well as respiratory tract secretions or urine; the same number of HA-MRSA strains, coming from patients hospitalised in various wards of the San Paolo Hospital, was collected from blood, respiratory tract secretions or other biological fluids. The DNA extracted was screened by PCR for PVL gene and by multiplex PCR to determine the mec type.
Results: Three MRSA harboured PVL gene, all belonging to CA-MRSA group (11.5%); two of them were SCCmec IV and one SCCmec V; the latter type was confirmed in a subsequent isolate four months later, differing only (data not shown) for the antimicrobial pattern (acquisition of erythromycin resistance). The PVL positive MRSA were isolated from cutaneous abscesses (two) and sputum. SCCmec IV was the predominant type among CA-MRSA (84.6%) and HA-MRSA (80.7%), followed by type I (7.7% CA-MRSA and 15.4% HA-MRSA), type III (3.8% both) and one type V among the CA-MRSA isolates. Interestingly, type I SCCmec isolates presented a multidrug-resistance phenotype characterised by gentamycin resistance.
Conclusion: As expected, PVL positive strains were found only among CA-MRSA and two out of three were associated to cutaneous abscesses. SCCmec type IV emerged as the most frequent genotype in this series of MRSA without any significant difference among the community or hospital acquired MRSA. To the better of our knowledge this is the first report on the molecular characterisation of Italian CA-MRSA isolates.

P1424 Analysis of meticillin-resistant *Staphylococcus aureus* isolates from catheter infections isolated in a large Italian hospital

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Objective: Twelve meticillin-resistant *Staphylococcus aureus* (MRSA) isolates from nosocomial catheter infections were studied. We determined their antibiotic susceptibility pattern (penicillin, oxacillin, erythromycin, tetracycline, clindamycin, telithromycin, gentamycin, ciprofloxacin, quinupristin-dalfopristin, rifampin, vancomycin and linezolid), and the presence of the genetic determinants of antibiotic resistance. To evaluate their genetic variability, isolates were subjected to multilocus sequence typing (MLST), SmaI macrorestriction/PFGE analysis and to staphylococcal chromosomal cassette/cassette chromosome recombinase (SCCmec/ccr) complex type determination.
Methods: The determination of the MICs was performed following the Clinical Laboratory Standards Institute guidelines. Genetic determinants of antibiotic resistance were screened by PCR, using specific primer set described in the literature, while a standard multiplex PCR protocol was used to assess the SCCmec and ccrAB complex type. MLST, SmaI macrorestriction of chromosomal DNA and PFGE analysis were performed according to standard procedures.
Results: An almost complete correlation was found between phenotypes and genotypic traits of antibiotic resistance. A major group of ten strains bore the SCCmec type I structure and showed the common sequence type st228. Among this group, only seven strains had a SmaI macrorestriction profile matching that of the archaic pandemic clone.

One strain, resistant to all antibiotics but vancomycin, was st239 and SCCmec type IIIA. However, its SmaI macrorestriction profile was not related to that of the pandemic clones having a type IIIA SCCmec.
It was not possible to assign the SCCmec type of the two remaining strains. They presented very rare multilocus sequence types, one being st83 and the other st395, and possessed SmaI macrorestriction profiles which were not related to any of the known pandemic clones.
Conclusions:

i. An extremely resistant strain belonging to st239 was isolated and characterised;
ii. in the st228 group, SmaI macrorestriction analysis was able to identify different subclones, showing an higher discriminatory power than MLST;
iii. the analysis of the SCCmec/ccrAB complex of two strains with rare sequence types was not possible with the multiplex PCR methodology used, indicating the possible presence of new variants of the SCCmec.

P1425 Molecular characterisation of meticillin-resistant *Staphylococcus aureus* strains isolated in a major general hospital in Italy over a three-year period

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Objectives: Meticillin-Resistant *Staphylococcus aureus* (MRSA) is the major cause of nosocomial infections worldwide. In the late 1990s highly virulent MRSA clones have emerged in the community, causing fatal infections in otherwise healthy people, without predisposing risk factors. Their characterisation showed a different genetic background on the staphylococcal cassette chromosome mec (SCCmec). Hospital- and Community-acquired MRSA (HA-, CA-MRSA) are different in genetic background, antibiotic susceptibilities and associated virulence factors. In order to investigate the presence of MRSA clones with the molecular characteristic of CA-MRSA in a large nosocomial setting, we typed all the MRSA isolated at the San Raffaele hospital (Milan) over a 3 year period.
Methods: 187 clinically significant MRSA strains were typed by multiplex PCR targeting unique and specific loci of SCCmec types I, II, III, IV and V, with concomitant mecA gene detection, the last used as a determinant of meticillin resistance and as internal positive control. All strains were tested for presence of the co-transcribed genes encoding for the Panton-Valentine leukocydin (PVL), lukS-PV and lukF-PV. Cluster investigation was performed by Pulsed-Field Gel Electrophoresis after SmaI restriction and analysed by InfoQuest FP software-version 4.5 (Bio-Rad).
Results: One hundred eight isolates (57.5%) carried SCCmec cassette typical of nosocomial strains: 100 harboured SCCmecI, 5 SCCmecII and 3 SCCmecIII. Fifty-eight isolates (31%) harboured one of the SCCmec associated to CA-MRSA: 57 were SCCmecIV and 1 was SCCmecV. Twenty-two strains (11.5%) were not typeable. Analysis of sensitivity patterns to selected antibiotics showed that CA-MRSA were more susceptible to non β-lactams than HA-MRSA. Two strains were PVL-positive: both carried SCCmecIV, were resistant to β-lactams only and were isolated from young patients without risk factors. Cluster analysis of MRSA harbouring SCCmecIV showed 2 major clades and 3 clusters of nosocomial transmission, one of them involving 5 patients. Cluster analysis of the HA-MRSA harbouring SCCmecI showed 11 major clades with 2 clusters of nosocomial transmission, one of them in Neurosurgical Intensive Care Unit, involving 19 patients from January to October 2007.
Conclusion: 57.5% of MRSA circulating in our hospital was identified as HA-MRSA; we report that 31% of the strains harbours the SCCmec cassettes associated to CA-MRSA although only 3.4% were PVL positive.

P1426 Looking back, current status, and future trends of MRSA clones in Italy

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Objectives: Our aims were i) to track the epidemiology of meticillin-resistance in Italy, studying the spread and prevalence of various MRSA clones over two decades; ii) to document MRSA changes in the phenotypic and genotypic features, and their shifts in the levels of susceptibility to glycopeptides; iii) to trace the origin of MRSA lineages in Italy comparing the genotypic backgrounds of contemporary isolates, with those of a sample of early MRSA strains from 1980.
Methods: Two hundred fifty-eight non-repetitive MRSA clinical isolates from various Italian hospitals between 1991 and 2006 were analysed for their antibiotic resistance, and typed by PFGE and SCCmec typing. All strains were grouped into clonal types, and representative isolates of the main clones were further characterised by MLST, generating the corresponding sequence type (ST)-SCCmec types for each MRSA genotype. A sample of twenty early MRSA strains from 1980 was also used for comparison.
Results: The most interesting feature was the increase of the Italian clone (ST228-I; PFGE E) over the last decade (from 27% during the first decade, to 70% during the second one), and its stability over this time; associated with the replacement of the multi-drug resistant and highly epidemic Iberian clone (ST247-I/IA; PFGE A), (46% during the first decade to 5% during the second one). The Rome clone (ST247-I/IA; PFGE C), one of the major local clones in the first decade (11%), has almost disappeared. ST1, ST15 and ST30 were the predominant earliest MLST types among the MRSA strains in 1980, carrying the first described SCCmec I, in which resistance to antibiotics, other than β-lactams, was very rare. A temporal shift in the susceptibility levels to glycopeptides was observed: vancomycin MIC90 value increased two fold from 1980 to 2006 (0.5–2 mg/L), while teicoplanin increased from 0.12 to 4–8 mg/L.
Conclusions: We describe the change of multi-resistance MRSA clones, which occurred in hospitals from 1991 to 2006, and the increase of the glycopeptide MIC levels, reflecting a worldwide trend, due to the abuse of such molecules in clinical therapy. We document the detection of ST1, ST15, and ST30 in the earlier isolates; we hypothesises a possible correlation of these strains with the current C-MRSA clones.

P1427 Molecular characterisation of meticillin-resistant and -susceptible *Staphylococcus aureus* from global clinical trials

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Objectives: To better understand the global epidemiology of *Staphylococcus aureus* we genotyped meticillin-resistant (MRSA) and -susceptible (MSSA) isolates recovered in 2004–5 from patients with uncomplicated skin infections in 10 countries during five Phase III clinical trials of retapamulin, a new topical antibiotic agent.
Methods: *S. aureus* isolates were tested for the presence of the Panton-Valentine leukocidin (PVL) genes. All 105 MRSA were analysed to determine the SCCmec type. All MRSA isolates, 118 PVL-positive MSSA, plus a representative subset of 69 PVL-negative MSSA were analysed by pulsed-field gel electrophoresis, and multilocus sequence typing (MLST).
Results: MRSA isolates were recovered mainly from the U.S. (60, 57%), India (27, 26%), Costa Rica (6, 6%), Germany (5, 5%), with the remainder (7, 6%) from South Africa, Russian Federation, France, and Peru. The most common MRSA strain (46, 44%), recovered exclusively in the U.S., was of the community-associated (CA) PVL-positive, ST8, USA300 type. Another four PVL-negative USA300 variants were recovered in Costa Rica and India. No pulsed field type USA300–0114

MRSA were found outside the U.S. Interestingly, a total of 10 PVL-positive and -negative USA300 isolates were found within the MSSA population from the U.S., South Africa, Peru, and Poland. USA800 was the second most common MRSA strain (11, 10%), primarily found in the U.S. and Costa Rica. PVL-positive MSSA isolates were from recovered South Africa (55, 47%), India (34, 29%), Russian Federation (12, 10%), U.S. (9, 8%), and Germany (6, 5%), with one isolate each from Canada and Peru. The most common PVL-positive MSSA strain was USA1200, primarily found in South Africa and the Russian Federation. This was followed by EMRSA15-related strains found only in South Africa and India. There was no predominant strain within the PVL-negative MSSA group with 29% of isolates exhibiting unknown types.

Conclusions: While results of this study are necessarily limited by the isolate numbers from countries in which patients were enrolled, the CA-MRSA strain USA300 was clearly associated with U.S. testing centres while no isolates of the major European ST80 CA-MRSA clone were recovered. In a number of instances genetic backgrounds associated with MRSA outbreaks were found in MSSA. The results underscore the importance of MRSA and MSSA surveillance to enhance understanding of the global spread and persistence of different *S. aureus* populations.

P1428 Genetic relatedness among European meticillin-susceptible and -resistant *Staphylococcus aureus* isolates from ceftobiprole clinical trials

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Objectives: Ceftobiprole (BPR) is an investigational parenteral cephalosporin that is unique from currently marketed cephalosporins in that it has broad-spectrum activity, including both meticillin-resistant *Staphylococcus aureus* (MRSA) and meticillin-susceptible *S. aureus* (MSSA). It is currently under clinical development for treatment of complicated skin and soft tissue infections (cSSSI) and pneumonia. The genetic relatedness among European MSSA and MRSA isolates collected from ceftobiprole cSSSI clinical trials was compared using spa typing and pulsed-field gel electrophoresis (PFGE).

Methods: Baseline isolates of MSSA [N=169; 73 were Panton-Valentine leukocidin (PVL) positive] and MRSA (N=74; 5 were PVL positive) from Eastern Europe (Bulgaria, Hungary, Latvia, Lithuania, Romania, Russia, Serbia & Montenegro, and Ukraine) were analysed. Spa typing and SCCmec typing (MRSA isolates) was done by PCR and/or DNA sequencing. PFGE was done on chromosomal DNA digested with SmaI. BPR MICs were determined according to CLSI methods.

spa type	MSSA (N)		MRSA (N)		No. of countries	BPR MIC (mg/L)	
	PVL+	PVL–	PVL+	PVL–		MIC_{50}	MIC_{90}
1	1	1	1	20	5	1	1
287	21	–	–	–	6	0.25	0.25
3	–	1	–	14	4	2	2
Unique	–	–	–	13	1	2	2
35	–	7	–	2	3	0.5	–
73	–	8	–	–	2	0.25	–
466	7	–	–	–	3	0.25	–
312	5	1	–	–	4	0.25	–
33	2	4	–	–	3	0.25	–

Results: There were 106 different spa types among all isolates analysed: MSSA PVL– (N=63), MSSA PVL+ (N=33), MRSA PVL– (N=17), MRSA PVL+ (N=3). Only nine spa types were found among two or more of the isolate groups. The nine most common spa types (Table) accounted for 44% (N=108) of isolates. The most prevalent MRSA clone had spa type 1 but PFGE patterns were not all the same; 57% had SCCmec type IV. Two MSSA isolates also had spa type I. Spa types 3 and “unique” were similar, differing by one motif. MRSA isolates with these spa types had SCCmec type III, similar PFGE patterns, higher BPR MICs (MIC50/90 of 2 mg/L) and were predominantly (78%) from Latvia. The most prevalent MSSA clone had spa type 287, similar PFGE patterns, and low BPR MICs (MIC50/90 of 0.25 mg/L); all were also PVL+.

Conclusion: There was little genetic similarity between MSSA PVL+, MSSA PVL–, MRSA PVL+, and MRSA PVL– isolates. From spa typing, MSSA PVL– isolates seemed the most heterogeneous and MRSA PVL– isolates the least heterogeneous. Ceftobiprole had excellent activity (BPR MIC90 of 2 mg/L) against all MSSA and MRSA isolates including those with the most common spa types.

P1429 The prevalence of carriers of meticillin-resistant *Staphylococcus aureus* in Copenhagen

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Objectives: The aim of the study was to elucidate the frequency of MRSA carriage in patients receiving treatment at the School of Dentistry at University of Copenhagen in order to achieve a greater knowledge of the carrier state of MRSA in a population of children and adults in Copenhagen.

Methods: During a period of one year from 01.10.06–01.10.07 1200 specimens from 600 patients were collected. A specimen from both nostrils and a specimen from both tonsils were taken from each patient, i.e. two specimens per patient. Each specimen was immediately plated on a 5% blood agar and inoculated into a nutrient selective broth. Both were incubated overnight at 37°C. At day two material from the broth was plated on a chrome agar plate and incubated overnight at 37°C. Susceptibility testing was performed at day three using a 10 μg cefoxitin disk (Oxoid, Basingstoke, UK) and semi-confluent growth on Iso-sensitest agar and incubated overnight at 37°C. Resistance was defined as $\leqslant$21 mm (SRGA). Cefoxitin resistant *S. aureus* were sent to the Staphylococcus Laboratory at Statens Serum Institut where further investigations including PCR for the mecA gene, spa typing and susceptibility testing were done.

Results: 518 adults $\leqslant$18 years with a mean age of 41.2 and a range of 18 to 91 years and 82 children $\leqslant$2 years with a mean age of 7.2 and a range of 2 to 16 years were included in the study. One child was positive for MRSA in the nose, the strain was a t022 spa type. The strain was additionally resistant to norfloxacin, but sensitive to erythromycin, tetracyclin, fusidic acid, rifampicin, streptomycin, kanamycin, linezolid and mupirocin. It was a healthy 13 year old boy born in Denmark of Danish parents. He had not been hospitalised in Denmark or abroad and he had not been travelling abroad for the last year. None of the adults were MRSA positive.

Conclusion: The prevalence of carriers of MRSA in Copenhagen seems to be very low and in this study only 0.2%. During the last year the number of patients with MRSA in Denmark has declined as a result of the implementation of national guidelines to prevent the disease from spreading in the hospitals and in the community.

P1430 Comparison of PFGE and spa for routine epidemiological typing to monitor and track spread of MRSA over time at a regional level

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Objectives: The epidemiology of meticillin-resistant *Staphylococcus aureus* (MRSA) is presently changing due to the worldwide emergence of community-associated MRSA (CA-MRSA). These clones are more diverse than the classical nosocomial MRSA clones. Pulsed-field gel electrophoresis (PFGE) of genomic macrorestriction fragments has been the gold standard method for epidemiological typing of MRSA. Recently, sequence-based typing methods such as sequencing of the polymorphic X region of the protein A gene (spa typing) have been commonly used due to their ease of interpretation, exportability and speed. For

infection control purposes we have collected, typed and stored all MRSA isolates detected in the west part of Sweden together with clinical and epidemiological data since 1983.We here report a comparison of PFGE with spa-typing for monitoring and tracking spread of MRSA over time.
Methods: The first detected CA-MRSA isolate of each PFGE-type (n = 38) was spa-typed, as well as the first isolate of all subsequent PFGE-subtypes of the nine most prevalent groups (n= 73). In addition all consecutive MRSA isolates January 2006 to June 2007 were typed by both methods. PFGE patterns were determined both visually, according to the Tenover criteria, and by computer matching with BioNumerics 5.0 software. Spa-typing was determined with Ridom StaphType software 1.4.11.
Results: For the first 38 isolates of each PFGE-type 25 different spa-types were found. The nine most prevalent PFGE-groups were found within 5 spa-groups according to BURP algorithm. The number of PFGE-subtypes, closely or possible related according to the Tenover criteria, within a group always exceeded that of the spa-types, and the relationship varied from 18:12 to12:2. For PFGE-groups belonging to sequence type (ST) 45 and ST30 PFGE and spa-typing resulted in concordant epidemiological results, in contrast to those consisting of ST80 and ST5. For isolates detected in 2006/07 the previous PFGE- and spa-types usually corresponded, with the exception of two PFGE-groups.
Conclusion: For the current circulating clones in our region, PFGE has a higher discriminatory power than spa-typing with the exception of the PFGE-groups belonging to ST45 and ST30. If only spa-typing had been used isolates of spa-type t002 (ST5) and t044 (ST80) as well as those of t008, t015, t037 and t127 would have mimicked outbreak situations which however not was the case according to the epidemiological investigation.

P1431 Clonal distribution of meticillin-resistant and susceptible *Staphylococcus aureus* invasive isolates in Belgium, EARSS/ SeqNet study, 2006–2007

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Objectives: To identify and determine spread of predominant clones of *S. aureus* causing invasive infection in Belgium during the 2006–2007 period.
Materials and Methods: As a part of the European Antimicrobial Resistance Surveillance System (EARSS)/ SeqNet survey, 23 Belgian hospitals were invited to collect $\geqslant$10 successive, non duplicate *S. aureus* isolates (5 MRSA and 5 MSSA) from normally sterile body sites during a 6 month period. PCR for detection of 16S/ nuc/ mecA and toxin genes (PVL/ TSST-1) were performed. Molecular typing was performed by spa sequencing using Ridom StaphType 1.4 (Ridom GMbH) and SCCmec type characterisation.
Results: A total of 202 *S. aureus* isolates including 91 (45%) MRSA and 111 (55%) MSSA were recovered from blood cultures (79%), puncture fluids (20%) and cerebrospinal fluids (1%). Spa typing showed 90 types clustered into 15 spa clonal complexes (CC) using the BURP algorithm. The majority of isolates belonged to spa CC008 (20%), CC740 (18%) and CC002 (15%) corresponding to major epidemic clones ST8, ST45 and ST5 by multi-locus sequence typing, respectively. Most (89%) MRSA isolates were classified in 4 clones, spa CC008-ST8-SCCmec IV (37%), spa CC740-ST45-SCCmec IV (34%), spa CC002-ST5-SCCmecII (10%) and spa CC002-ST5-SCCmecIV (8%). In contrast, MSSA isolates showed a wide diversity and belonged to 15 spa CC, each including 2 to 13 isolates. Among MSSA isolates 20% harboured the 3 major MRSA spa CC and 28% of MSSA isolates were classified into 3 spa types, CC084, CC015 and CC078 not found in MRSA isolates. Toxin gene analysis revealed 2 MRSA isolates carrying PVL genes and belonging to ST59 and ST8. TSST-1 gene was detected in 7 MRSA isolates belonging to CC002-ST5-SCCmec IV.
Conclusions: As found in previous surveys in Belgium, 4 major genotypes predominated among MRSA isolates. In contrast, MSSA isolates showed a wide genotype diversity, with sharing of common genotypes with major MRSA strains. PVL and TSST-1 were recovered in 2% and 8% of MRSA isolates, respectively.

P1432 Phage types versus spa types among MRSA strains isolated in a Belgian hospital (2002)

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Staphylococcus aureus represents a growing public health burden owing to the spread of nosocomial multi-resistant clones and to the emergence of community-associated meticillin-resistant *S. aureus* (MRSA). In Belgium, the proportion of MRSA was estimated at 31% in 2005 (EARSS). Strain typing of pathogens is essential to pinpoint the sources and routes of transmission and to forecast future trends. Phage typing has been one of the first standardised typing techniques for *S. aureus* (International Union of Microbiological Societies). Recently, spa genotyping has proven to be an effective typing tool to distinguish strains within a heterogeneous species such as *S. aureus*. A link between phage types and spa types among Belgian MRSA isolates was investigated.
In 2002, from a 487-bed general hospital located in northern Belgium, 159 isolates were received by the National Phage Typing Centre. After MRSA confirmation (coagulase test, minimum inhibitory concentration of oxacillin and mecA gene determination) 150 MRSA were further examined.
[J]* phage types (= lysis only by group III phages) characterised 45% of them, 12% belonged to a local phage type 29/42E/54/D11*, 41% were not assigned to a defined group. Three isolates could not be phage typed. Twelve different spa types (Ridom StaphType) were found: 39% belonged to t038, 27% to t121, 14% to t041, 5% to t655, and 4% to t002 and t024 each. Two spa types were only sporadically represented (2 and 3 isolates) and four were unique to one isolate. The t041 spa type was specific for the 29/42E/54/D11* phage type. The majority of the t121 typed isolates were related to a [J]* phage type, namely the J2* type (only lysed by phage 54). This was also the case for all our isolates belonging to the t024 spa type. Within the t038, t655 and t002 spa types a diversity of phage types was found.
In conclusion, some concordance was found between spa and phage typing of MRSA. Nevertheless, the combination of both methods allowed better discrimination among about half of the isolates.

P1433 Spread of MRSA clones in German hospitals and in vitro activity of tigecycline

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Objectives: MRSA are now a leading cause of healthcare associated infections. More recently, MRSA have also established as a significant community-associated pathogen. In Germany, the prevalence of MRSA is about 20% of all *S. aureus*. MRSA are often multi-resistant, which has limited the number of therapeutic options. Vancomycin (VAN) has been considered as the drug of choice, but new agents are required due to the emergence of strains with reduced susceptibility to glycopeptides. Recently, tigecycline (TGC) has become approved for the treatment of some MRSA infections. The objective of this study (G-TEST) was to evaluate the distribution of MRSA clones among *S. aureus* isolates collected in 15 laboratories and to determine the vitro activity of TGC and other antimicrobials against these strains.
Methods: From April to August 2005, each laboratory was requested to collect 10 MRSA from hospitalised patients. Isolates were characterised by spa sequence typing according to the SeqNet.org protocol. MICs of TGC, doxycycline (DOX), moxifloxacin (MFX), gentamicin (GEN), linezolid (LZD), and VAN were determined in a central laboratory by the microdilution method according to the German DIN standard. EUCAST breakpoints were applied to interpret MICs, as available.
Results: Of the 155 MRSA collected, 154 were analysed by spa typing. 65% of the MRSA belonged to MLST types ST5/ST225 (spa

types t002/t003; n = 33) und ST22 (spa types t022/t032; n = 37). Either epidemic type was found in 14 laboratories. ST22 was predominant in Northern Germany and in the Berlin area, while ST5 and ST225 were widely distributed in Western Germany. ST228 was predominant in Southern Germany. Eight isolates belonged to the ST8 clone, one of which carried the Pantone-Valentine-leucocidin gene (lukS-lukF). This strain was identical to the caMRSA clone USA300, as determined by additional PCR analyses (arcA, msrA, mphB). One isolate belonged to the caMRSA clone ST80, which is widely disseminated in Europe. 77% of isolates were susceptible to GEN, but only two (1%) were susceptible to MXF. Ten isolates showed reduced susceptibility to DOX (MIC > 1 mg/L). Of these, three belonged to spa type t037 (ST239). All MRSA were susceptible to VAN, LZD and TGC.

Conclusion: TGC demonstrated excellent in vitro activity against MRSA isolates recently recovered from hospitalised patients in Germany. Therefore, within its range of clinical indications, TGC could be of potential use for the treatment of MRSA infections.

P1434 Molecular epidemiology of meticillin-resistant *Staphylococcus aureus* in fourteen cities in China from 2005 to 2006

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Objective: To investigate the prevalence, antimicrobial resistance pattern, and the molecular epidemiology of MRSA in multiple centres of China from 2005 to 2006, and to evaluate the antimicrobial activities of tigecycline, daptomycin, and ceftobiprole against MRSA.

Methods: From 2005 to 2006, 1529 consecutive and non-repetitive *Staphylococcus aureus* were collected from 14 cities. The MICs of antibacterial agents were determined by agar dilution. The genotypes of SCCmec were determined by multiplex PCR. Pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) were used to type MRSA. Panton-valentine leukocidin (pvl) gene was detected by PCR for all *S. aureus*.

Results: The mean prevalence of MRSA was 50.4% (408/809) in 2005, and 43.9% (315/717) in 2006, respectively. Shanghai has the highest MRSA prevalence (74.0%), followed by Qingdao (69.8%), Xi'an (68.0%), and Dalian (61.5%). The prevalence of MRSA was the lowest in Changchun (24.2%), Nanjing (25.4%), Shenzhen (22.4%) and Urumchi (29.2%). Only 4.2%-12.6% of MRSA were susceptible to erythromycin, quinolones, gentamicin, or tetracycline. About 78.6% of isolates were susceptible to trimethoprim/sulfamethoxazole. All isolates were susceptible to teicoplanin and vancomycin. Ceftobiprole, daptomycin, tigecycline, and linezolid had excellent activities with MIC50 and MIC90 of 2, 2 mg/L; 0.5, 0.5 mg/L; 0.125, 0.25 mg/L; 1, 2 mg/L, respectively. SCCmec type III and II accounted for 66.1% (478/723) and 14.1% (102/723), respectively. However, in Shenyang and Dalian, SCCmec II was more common than type III. PFGE typing found 24 different patterns. Clone A accounted for 50.1%, existing in 12 cities and clone R for 23.5%, and existing in 9 cities. Six sequence types (ST) were found in 53 representative isolates, with ST239 accounting for 75.5% and ST5 for 17.0%. The prevalence of pvl gene in all *S. aureus* was 9.4%. Two isolates from Guangzhou in 2006 belonged to community-acquired MRSA (CA-MRSA), one with ST88-SCCmec IV, the other one with ST59-SCCmec IV.

Conclusions: The prevalence of MRSA differed in areas in China. The most predominant types of MRSA in China were ST239-SCCmecIII and ST5-SCCmecII, which indicated that several international epidemic MRSA clones might exist in China. Clone spread resulted in the higher prevalence of MRSA in big teaching hospitals. CA-MRSA was uncommon in China. Ceftobiprole, daptomycin, tigecycline and linezolid showed excellent activities against all MRSA tested.

P1435 spa sequence typing of meticillin-resistant *Staphylococcus aureus* isolated in Bulgaria, 2005–2007

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Objectives: The aim of the present study was to define the most prevalent spa types of MRSA isolated in Bulgaria in the period 2005–2007 and to associate them with the corresponding SCCmec type, PVL production and phenotype of antibiotic resistance.

Methods: A total of 224 MRSA isolated between 2005 and 2007 in 19 hospitals located in 14 cities in Bulgaria were included in the study. Community-acquired MRSA isolates were also included. The isolates were grouped according to their resistance phenotypes and 85 representative isolates were selected for further genotyping, including spa sequence typing, SCCmec typing and PCR detection of the genes encoding PVL.

Antibiotic susceptibility testing was performed according to CLSI. Meticillin-resistance was confirmed by latex agglutination test for PBP 2a and PCR detection of mecA gene. SCCmec typing was performed using multiplex PCR protocol. The presence of PVL genes were assessed by PCR. The isolates were further analysed by spa sequence typing, according to SeqNet.org protocol, spa types were designated using Ridom Staphtype software.

Results: Among the investigated isolates 18 spa types were defined: t037, t030, t010, t1143, t045, t2168, t1507, t044, t416, t731, t008, t024, t005, t015, t078, t1194, t1368 and t1531. The most prevalent spa type was t037 possessing SCCmec type III, associated with the resistance phenotype K, Gen, Tet, Er, Cli (MLS-const), Cip, SXT, followed by t030 with SCCmec type III (K, Gen, Tet, Er, Cli (MLS-induc/MLS-const), Cip, Rif) and t010, SCCmec type IV, which showed resistance to oxacillin only. The isolates belonging to these spa types were negative for PVL genes.

Six cases of infections caused by community-acquired MRSA (cMRSA) were defined. MRSA isolates from 5 patients belonged to spa type t008 (K, Er, Tet, Cip) and from one patient to spa type t044 (K, Tet, Fus). Both t008 and t044 strains were found positive for PVL and possessed SCCmec type IV.

Conclusion: In the present study, the most prevalent spa types in the Bulgarian hospitals were t037 and t030, SCCmec type III, frequently associated with ST239 (Brazilian/Hungarian) clone, followed by t010, SCCmec type IV, which possibly belongs to ST5-IV (Paediatric) clone. Most of the cases of PVL-positive cMRSA infections were caused by t008, SCCmec type IV, which corresponds to the USA300 strain.

P1436 Molecular characterisation of *Staphylococcus aureus* isolates belonging to two most prevalent spa types recovered from Romanian hospitals during 2006–2007

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Objectives: In this study, a molecular analysis was undertaken in order to further characterise *Staphylococcus aureus* clinical isolates belonging to two most prevalent spa types: t030 and t127 that were identified during 2006–2007, in Romania.

Methods: A total of 91 *S. aureus* isolates collected from hospital in-patients were examined phenotypically and subtyped using a single-locus sequence typing method, namely spa typing. This method is based upon the polymorphism of *S. aureus* protein A gene, whose repeats are variable in number and individual sequence. Isolates belonging to spa types t030 and t127 were selected for further molecular characterisation and cluster analysis of the SmaI patterns, generated by pulsed-field gel electrophoresis. Meticillin-resistant *S. aureus* (MRSA) isolates were confirmed by PCR targeting mecA gene. Staphylococcal chromosomal cassette mec (SCCmec) types were determined by performing a multiplex PCR with specific primers for the key genetic elements. The presence of lukS-lukF genes encoding Panton-Valentine leukocidin (PVL) and other toxin genes was tested by PCR.

Results: spa type t127 comprised 26 *S. aureus* isolates (28.5% of all examined isolates) and spa type t030 comprised 18 isolates (20%), respectively. All t030 isolates were MRSA with mecA gene carried on a type III SCCmec genetic element. Twenty-one out of 26 t127 isolates were MRSA and harboured a type IV SCCmec structure. Different virulence gene profiles were identified, but all the 44 isolates were PVL and seb genes negative, irrespective of spa type. Clonal relationships were established within each spa type group of isolates.
Conclusion: We have found that the prevalent spa-types of *S. aureus* isolates recovered from Romanian hospitals in 2006 and 2007 were spa-type t030 associated with SCCmec type III structure and spa-type t127 associated with SCCmec type IV structure. PFGE profiles showed that the isolates which share each of these types belong to the same clone. More detailed studies, including superantigen genes detection, will be performed, in order to further characterise the isolates belonging to these prevalent spa-types.

P1437 Changing molecular epidemiology of *Staphylococcus aureus* in Korea over a ten-year period

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Objectives: Most studies of molecular epidemiology on *Staphylococcus aureus* in Korea have been limited to meticillin-resistant *S. aureus* (MRSA) isolated in tertiary hospitals. This study was performed to evaluate the changes of molecular characteristics of *S. aureus* isolated both in the community and in hospitals over a 10-year period.
Methods: Antibiotic susceptibility tests, staphylococcal cassette chromosome mec (SCCmec) typing, multilocus sequence typing (MLST), and toxin gene analyses by multiplex polymerase chain reactions were performed on *S. aureus* isolates collected from clinical specimens from 1996 through 2005.
Results: Four-hundred thirty-three *S. aureus* isolates were tested, which included 148 meticillin-susceptible *S. aureus* (MSSA) and 285 MRSA. The clonal complex 1 strain, including the sequence type 1 (ST1) strain, was prevalent (29%, 28/96) in MSSA until 2003, but MLST profiles of MSSA showed a great diversity. While ST72, the most common strain (ST72-MRSA-SCCmec IV/IVa) of community-associated MRSA (CA-MRSA) in Korea as previously reported (J Antimicrob Chemother 2007;60:1108–14), was found in only one MSSA isolate till 2003, it has been increasing not only in MSSA (5/31) but also in MRSA (9/15) from the anterior nares of healthy people since 2005. Moreover, ST239-MRSA-SCCmec III/IIIa has surpassed ST5-MRSA-SCCmec II/IIa, the most prevalent strain till 2003, among MRSA isolates from the hospitals since 2005. Toxin gene profiles also showed a diverse distribution, but certain profiles were associated with meticillin resistance and the SCCmec types. Pvl genes were detected in only 7.4% of the total MSSA and 1.1% of MRSA during the study period.
Conclusion: The epidemiology of *S. aureus* in the community and hospital settings has changed gradually during the past 10 years in Korea. ST72-MRSA-SCCmec IV/IVa emerged recently in the community, even if it is uncertain whether this is a new clone or from the hospitals.

P1438 Meticillin-resistant *Staphylococcus aureus* – Community-acquired phenotype spread in hospitals in Bogotá, Colombia

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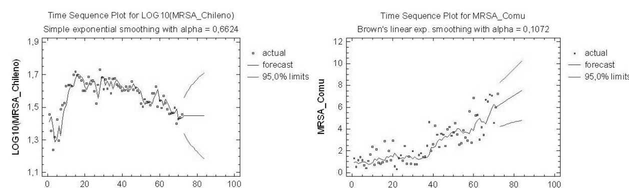
Introduction: Meticillin Resistant *Staphylococcus aureus* (MRSA) has been largely known as a nosocomial microorganism with clonal dissemination. In Colombia, the Chilean clone has replaced the previous one as the most common cause of nosocomial infections in hospitals. Recently, isolates of community acquired MRSA (CA-MRSA) has been identified in Bogotá. The prevalence of CA-MRSA in colombian hospitals hasn't been reported. Here we show the observed change in the frequency of some phenotypes related to the Chilean and CA-MRSA clones and present a forecast for their behaviour.
Methods: Time-series analysis of MRSA isolates of 23 tertiary hospitals of the GREBO network between 2001 and 2006 was done. Microbiological data from hospitals were monthly transferred and compiled in the GREBO data base. A descriptive analysis of isolates susceptibility pattern was done with the Whonet (ver. 5.4) software. Two time-series anlalysis modelling were done by use of the Box-Jenkins methodology (72 monthly periods): One with the Chilean phenotype (Oxacillin, ciprofloxacin, erythromycin, clindamycin resistant, susceptible to cotrimoxazole, rifampin and tetracyclines) and one with the CA-MRSA phenotype (Oxacillin resistant susceptible to the others).
Results: 33156 *S. aureus* isolates were identified in the network. Annual frequency of MRSA starting 2001 was 38%, 46%, 45%, 45%, 38% and 34%. CA-MRSA phenotype showed an increase from 1% in 2001 to 5.4% in 2006. The Chilean clone series was best described by a simple exponential (alpha = 0.6677) and for the CA-MRSA phenotype the Brown lineal model fit better. The forecast for the Chilean clone phenotype doesn't show a clear cut trend (high confidence intervals). On the opposite, the forecast for the CA-MRSA phenotype series shows a high increase for the followingf year.



Time series and forecasting for Chilean and community-acquired MRSA clone.

Conclusions: CA-MRSA phenotype showed a sharp increase in the last years and the forecast predicts a higher percentage for the following year. The chilean clone seems to have a decrimental trend, but it is not as clear. This finding has a big impact on public health measures and medical education.

P1439 An outbreak of meticillin-resistant *Staphylococcus aureus* (spa type t008) in the paediatric intensive care unit of a university hospital, Tartu

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Objectives: In Tartu University Hospital the prevalence of meticillin-resistant *Staphylococcus aureus* (MRSA) has been low (1–4%) until now. In November 2006 the routine surveillance of clinical microbiology laboratory reports revealed a case of MRSA infection in the paediatric intensive care unit (PICU). The aim of the study was to describe the outbreak of MRSA in PICU and to detect the genetical relatedness and protein A type of the consecutive MRSA isolates.
Methods: During the outbreak period, from November 27, 2006 to July 29, 2007, a total of 20 nonduplicated MRSA isolates from clinical and surveillance samples were obtained. Antibiotic susceptibility was measured by disk-diffusion method, meticillin-resistance was confirmed by the detection of mecA gene using PCR. Clonal relatedness of the isolates was performed with Pulsed Field Gel Electrophoresis (PFGE) and sequencing of the polymorphic region of protein A (spa typing). Interventions to control the outbreak included active surveillance, contact isolation of infants, decolonisation of healthcare workers (HCWs) and retraining in hand hygiene.
Results: The first MRSA strain of the outbreak was isolated from the eye secretion of a premature baby (birth weight 960g) with congenital sepsis on his 2nd day of admission. Patient's mother had been treated for osteomyelitis before giving birth. During the outbreak 17 patients among the total of 228 admissions were affected. There were 4 neonates with bloodstream infection, 8 had noninvasive disease (skin and soft tissue infection, pneumonia, conjunctivitis) and 5 were colonised with

MRSA. The majority of patients were premature neonates. The median time from admission to acquisition of MRSA was 6 days (range 1–26). Three of the 106 HCWs were colonised with MRSA. MRSA was not detected from the environmental samples. 60% of MRSA isolates were susceptible to clindamycin, gentamicin and ciprofloxacin, and all to trimethoprim-sulfamethoxazole and vancomycin. All MRSA strains showed closely related PFGE pattern and belonged to the spa type t008. Colonised HCWs were treated with topical mupirocin and hexachlorophene showers which ended the outbreak.

Conclusion: This is the first reported outbreak of MRSA spa type t008 (commonly found in USA and Europe) in Estonia. We speculate that the primary source of this outbreak was the mother of the index patient and secondary sources were colonised HCWs. Routine surveillance for MRSA has now been implemented in the PICU.

P1440 Meticillin-resistant *Staphylococcus aureus* clones recovered from bloodstream infections in Czech hospitals in 2000–2007

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Objectives: Czech isolates of meticillin-resistant *Staphylococcus aureus* (MRSA) associated with invasive disease were characterised by spa typing. The aim of the study was to determine the clonality of MRSA isolates prevalent in Czech hospitals.

Methods: Thirteen national microbiology laboratories participating in the European Antimicrobial Resistance Surveillance System (EARSS) submitted to the National Institute of Public Health the first 5 consecutive MRSA isolates collected from individual patients in each hospital in six-month sampling periods from September to February in 2000 through 2007. The screening of resistance to oxacillin was performed using Oxoid Chromogenic MRSA Agar. The minimal inhibitory concentrations (MICs) of oxacillin, erythromycin, clindamycin, chloramphenicol, gentamicin, tobramycin and vancomycin were determined by the CLSI broth microdilution method. All MRSA isolates (n = 140) were tested for the presence of the mecA gene and spa typed. Detection of Panton-Valentine leucocidin (PVL) genes was performed in 56 isolates.

Results: The number of MRSA isolates per participating laboratory ranged from 6 to 19. Spa typing revealed 23 different spa types; however, only 7 spa types were identified in at least 2 isolates. The most prevalent spa types were as follows: t003 (n = 72), t030 (n = 23), and t032 (n = 12). Since the first isolation in 2002, spa type t003 has gradually replaced spa type t030 that was highly prevalent in the Czech Republic until 2002–2003. The proportion of spa type t003 increased from 0% in 2000–2001 to 67.6% in 2006–2007 while that of spa type t030 decreased from 62.5% to 0.0%, respectively. PVL genes were found in a single isolate of spa type t044.

Conclusion: A significant shift in the prevalence of spa types was observed. Spa type t030 (Brazilian clone) has been replaced by spa type t003 (Rhine Hesse clone). The presence of EMRSA-15 clone of spa type t032 was also detected in 4 Czech hospitals.

P1441 Community-associated meticillin-resistant *Staphylococcus aureus* in Finland: ten-year perspective

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Objectives: During 1997–1999, one fifth of all Finnish meticillin-resistant *Staphylococcus aureus* (MRSA) isolates (n = 308) were obtained from persons without connection to hospitals, and three strain types (FIN-4, -11, -14) were associated with community acquisition. Thereafter, the number of MRSA isolates has remarkably increased and new strain types appeared. We estimated the proportion of community-associated MRSA (CA-MRSA) and compared the MRSA isolates obtained during 2004–2006 in persons with and without hospital contact in terms of strain type. The results were compared with those reported from years 1997–1999.

Methods: MRSA isolate was defined as community-associated if MRSA specimen was obtained outside hospital settings or within two days of hospital admission from a person who had not been hospitalised within two years before date of MRSA isolation. We analysed previous hospitalisations for all MRSA-positive persons during 2004–2006 (n = 3054) by using data from the National Hospital Discharge Register. The isolates were typed by pulsed field gel electrophoresis and tested for antimicrobial susceptibility. Selectively, multi locus sequence typing (MLST), spa typing, SCCmec determination, and Panton-Valentine leukocidin (PVL) gene detection were performed.

Results: Of the 4054 MRSA-positive persons, 4030 (99%) were included in the study; 570 (14%) had CA-MRSA. A total of 36 strain types were shared by $\leqslant$5 persons, and 109 sporadic strains were identified. Ten different strain types (FIN-4, -5, -11, -12, -15, -17, -19, -20, -25, -30) as well as sporadic strains were more likely to be found from persons without hospital contact. All 10 CA-MRSA strain types had SCCmec IV or V, representing 8 different MLST types and 9 spa types, and 4 were PLV-positive. Of the sporadic CA-MRSA strains, 16 were tested for PVL genes, and 3 were positive.

Conclusions: The proportion of CA-MRSA did not increase. However, the absolute number of CA-MRSA isolates rose five-fold. Two of the old CA-MRSA strain types persisted and several new emerged. Internationally recognised, PLV-positive CA-MRSA clones were also recognised in Finland.

P1442 Evaluation of the resistance profile and detection of virulence genes in *Staphylococcus aureus* isolates from clinical and surveillance samples collected at a Brazilian teaching hospital

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Objectives: This study aimed at evaluating the resistance profile and presence of the gene mecA and enterotoxin genes sea and sed, as well as toxic shock syndrome toxin-1 (TSST-1) in *Staphylococcus aureus* samples isolated from clinical and surveillance samples from patients of Bauru State Hospital, Brazil.

Methods: Fifty-two *S. aureus* samples isolated between October/2006 and March/2007 were included. Oxacillin resistance was assessed by drug diffusion test as recommended by CLSI, using oxacillin (1 μg) and cefoxitin (30 μg) disks. Genes mecA, sea, sed and tst were detected by PCR.

Results: Of the 52 samples analysed, 29 (55.8%) were resistant to both disks tested, as confirmed by the presence of the mecA gene, and 8 (15.4%) showed colony growth within inhibition halos, suggesting heteroresistance. These colonies were identified and retested with oxacillin and cefoxitin disks, and PCR was once more performed for the amplification of the gene mecA, which confirmed the presence of oxacillin-resistant(MRSA) heteroresistant *S. aureus*. The detection of toxin genes revealed that one of the genes studied was found in 8 samples (15.4%) while the sea gene was present in 6 (11.5%), and genes tst and sea were concurrently seen in two (3.8%) of the samples. Of the two samples exhibiting genes sea and tst, one was resistant to oxacillin and the other was heteroresistant. Of the six sea-positive samples, two were MRSA and one was heteroresistant. These results were confirmed by the amplification of the mecA gene.

Conclusion: These findings highlight the importance of the genotypic evaluation of MRSA presence, and demonstrate the relationship between the presence of virulence genes and oxacillin resistance, which may result in the dissemination of these multiresistent toxigenic strains among patients hospitalised in this part of Brazil.

Financial Support: FAPESP

P1443 Community-associated meticillin-resistant *Staphylococcus aureus*: prospective surveillance of prevalence and clinico-epidemiologic feature among patients with otorrhoea in primary Korean otolaryngology clinic

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Objectives: Community-associated meticillin-resistant *Staphylococcus aureus*(CA-MRSA) has emerged as an important infection in the community setting. Most of CA-MRSA infections in Korea are ear infection(acute ear infection, chronic otitis media), but little is known about the epidemiology of CA-MRSA in primary otolaryngologic clinics. To determine the prevalence, and clinical and microbiological characteristics of CA-MRSA in ear infection of community setting, we designed a prospective survey. We also investigated the analysis of clonal relatedness of CA-MRSA from five different clinics.

Methods: From January to June, 2007, 178 otorrhoea samples were collected from five clinics in west area of Seoul and were classified MRSA or not. A comparative analysis of risk factors for CA-MRSA infection was performed. SCCmec typing by multiplex PCR, molecular typing by pulsed-field gel electrophoresis(PFGE) and multi-locus sequence typing(MLST) were done with MRSA isolates.

Results: Proportion of MRSA was 36.7%(33/90) and was significantly different between 5 clinics($p < 0.01$). Recent antibiotic use was most significant risk factor for MRSA infection($p < 0.01$). SCCmec type II isolates were most prevalent(63.6%, 21/33) and type IV isolates were 21.2%(7/33). 8 MLST types were discovered. Among them, 4 types(ST83, 639, 509, 627) were newly discovered in Korea. Same PFGE type was discovered in different clinics. Some MRSA isolates had a similar or same PFGE types in clinic of high MRSA rate.

Conclusion: This study demonstrates that CA-MRSA infection was prevalent and variable MRSA clones were epidemic in otolaryngologic clinics of community. Most of MRSA isolates had molecular characteristics of hospital-associated MRSA(HA-MRSA) and some others had molecular characteristics of CA-MRSA.

P1444 Prevalence of meticillin-resistant *Staphylococcus aureus* among patients undergoing peritoneal dialysis in Taiwan

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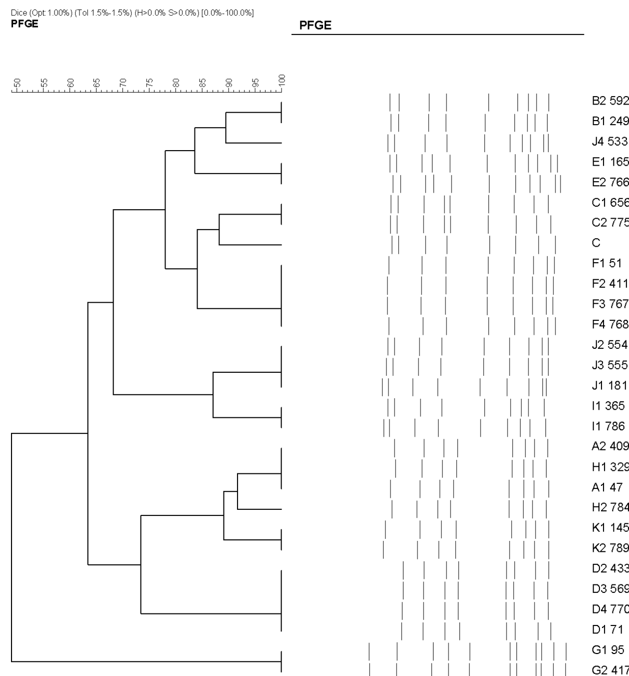
Objectives: The aim of current study was to investigate the prevalence of meticillin-resistant *Staphylococcus aureus* (MRSA) infection among patients receiving peritoneal dialysis (PD) and molecular characteristics of the MRSA isolates.

Methods: From January to November, 2007, 177 patients undergoing PD at our hospital were screened for MRSA infection using cultures of the nares and the PD exit site as well as real-time PCR assay (BD, Geneohm). Swab culture of both sites was repeated one month later for each patient. All the MRSA isolates were studied on their genetic correlation by pulse-field gel electrophoresis (PFGE). The SCCmec element typing and the Panton-Valentine leukocidin (PVL) gene were evaluated in all isolates. A carrier was defined as a patient with indistinguishable MRSA clones isolated from two different time periods regardless the isolation sites. A coloniser was defined as a patient with MRSA isolated from only one of the two different survey cultures regardless the isolation sites.

Results: During the study period, 7 patients (3.9%) were identified as carriers and 7 patients (3.9%) as colonisers. A total of 29 MRSA isolates were obtained from the two surveys of the nares and exit site. Compared with nasal swab cultures, the sensitivity and specificity of real-time PCR assay in the detection of MRSA infection was 90% and 98.8%, respectively. There were 6 pulsotypes identified (pulsotypes A to F) with no predominant clone. Three pulsotypes (pulsotypes B, C, and D) contained SCCmec type IV. Two pulsotypes (pulsotypes A and E) possessed SCCmec type V and were the only pulsotypes that had PVL gene. After follow-up for 9 months, one of the 7 carriers developed MRSA-related exit site infection and none had peritonitis. Of the 7 colonisers, one developed MRSA-related exit site infection and another one *Escherichia coli*-related peritonitis.

Conclusion: We found that the prevalence of MRSA infection among patients undergoing PD was similar to that of community population (3.5%) and was much lower than that of patients undergoing hemodialysis (5.9%) in a previous survey in Taiwan.



P1445 Longitudinal carriage of *Staphylococcus aureus* in families of non-healthcare workers in Hong Kong

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Objectives: Overall 25% of humans carry *S. aureus*, but this colonisation may be transient or persistent, and a high proportion is never colonised. Transmission of *S. aureus* has been documented in families of healthcare workers (HCW). This study investigated longitudinal carriage and transmission of *S. aureus* in families without HCWs.

Methods: Nasal samples from 100 non HCW associated families, each with at least three persons living together, were obtained at monthly intervals for 3 months. Swabs were cultured on blood agar, mannitol salt agar (MSA), MSA with 6gg/ ml oxacillin, and enriched in brain heart infusion broth with 5% salt. Colonies showing staphylococcal morphology were confirmed as *S. aureus* using Staphaurex. Susceptibility to oxacillin and cefoxitin and other antibiotics was determined by disc diffusion. Strains appearing meticillin resistant were examined for the presence of mecA by PCR, and characterised for PVL gene carriage, SCCmec and agr type.

Results: Of 342 subjects, 27.4%, 24.9% and 19.3% were colonised on the first, second, and third sampling respectively. Overall, 11.4% were colonised persistently, 26.9% transiently, and 61.7% never colonised. Persistent carriage was more common in those aged <10 and >60. Only non-carriers were observed in 29 families and two families consisted only of persistent carriers. Mothers were the most frequently colonised family member. Of 57 families with at least one colonised member on the first occasion, 16 had at least one other family member with the same strain (12 of 57 families on the second, and 10 of 48 on the third). Between first and second, and second and third samplings, in 7 and 6 of the colonised families respectively, another family member had become colonised with the same strain on the subsequent sampling. MRSA was isolated from 4 subjects in the first collection, including a mother and son with the same strain resistant to β-lactams only (SCCmec type IV variant). The second collection yielded only one MRSA with SCCmec type IV, but on

the third, 3 subjects were MRSA-colonised, including a different mother and son pair with a type IV variant displaying erythromycin resistance. All MRSA strains were agr type I and none harboured PVL genes.
Conclusions: Transmission occurred in 10 ± 12% of families over a one month period. Although MRSA colonisation remained low in families without HCWs (range 0.3–1.1%), transmission of MRSA between a mother and son occurred on two occasions.

P1446 Panton-Valentine leukocidin gene and staphylococcal chromosomal cassette mec types among clinical isolates from patients at a Khon Kaen hospital, Thailand

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Background: Increasing interest in Panton-Valentine leukocidin (PVL)-producing *S. aureus*, due to its involvement in skin and soft tissue infections and severe necrotising pneumonia have been reported worldwide. Recently, we reported first case of PVL producing *S. aureus* in an HIV patient with a fatal outcome. However, little is known about PVL producing *S. aureus* among isolates in Thailand.
Objective: The purpose of this study was to determine the frequency of PVL gene and staphylococcal chromosomal cassette mec (SCCmec) types among clinical isolates of *S. aureus* obtained from patients at Srinagarind hospital, Thailand, and also to assess the genetic relatedness of PVL positive strains.
Method: We used PCR method to test for the PVL gene in 316 clinical isolates of *S. aureus* obtained from clinical samples between September 2004-March 2005. For all PVL positive *S. aureus* strains, we analysed by RAPD method using OPE 20 and P1 primers to assess the genetic relatedness. MRSA were detected by oxacillin disc diffusion method and were confirmed by the precence of mec A gene by PCR method. The SCC mec types of the MRSA isolates were performed by multiplex PCR assay.
Result: A total of 316 strains (136 MSSA strains and 180 MRSA strains were analysed. Twenty eight strains of MSSA were found to harbour the PVL gene. PVL positive *S. aureus* were 8.86% of all *S. aureus* strains analysed. Compared with MSSA alone, PVL positive strains comprised 20.59%. The RAPD analysis differentiated the PVL positive *S. aureus* isolates into 4 to 7 distinct genotypes, according to the OPC-20 and P1 primer. All the PVL positive isolates had the same antibiogram pattern, they were susceptible to cephalothin, cotrimoxazole, clindamicin, erythromycin and oxacillin. None of MRSA strains were positive for PVL gene and only SCC mec type III and subtype IIIa and IIIb were found among Thai isolates
Conclusion: Our study found high percentage of PVL positive *S. aureus* strains. All MRSA isolates carried the SCC mec type III complex and did not harbour the PVL genes. The incidence and clinical significance of PVL positive *S. aureus* in Thailand needs further investigation.

P1447 Meticillin-resistant *Staphylococcus* in Bahrain: evidence of MRS transmission and co-carriage of vancomycin-resistant enterococci

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Objectives: To determine the transmission of meticillin resistant Staphylococci (MRS) between the hospital and community and evaluate the levels of co-colonisation of MRS positive patients with vancomycin resistant Enterococci (VRE) in our institution
Materials and Methods: From November 2005-April 2006, meticillin resistant *Staphylococcus aureus* (MRSA) and meticillin resistant coagulase negative *Staphylococcus* (MRCoNS) positive patients at the Salmaniya Medical Complex, Kingdom of Bahrain were studied. Patients stool specimens were investigated for Enterococci on bile-esculin-azide agar. Enterococci were identified to species level using API 20 STREP, BD PHOENIX system and PCR. Vancomycin resistance in enterococci isolates was confirmed by PCR. Nasal and axillary swabs obtained from family members of patients were screened for MRS. The relatedness of the MRS strains from matching pairs of patients and family members was determined using pulsed-field gel electrophoresis (PFGE).
Results: In total, 182 MRS positive patients (93 MRSA, 89 MRCoNS) and 356 family members were studied. Thirteen MRS patients also carried VRE (12 MRSA & 1 MRCoNS). These were predominantly *E. gallinarum* with vanC1 genotype and one strain was *E. faecium* (vanB genotype). Two *E. gallinarum* isolates haboured an additional vanB gene. Seven MRSA and 41 MRCoNS strains were isolated from the family members. PFGE analysis revealed the presence of variants of a single MRSA clone among patients and their relatives. Twelve MRSA isolates were identical or closely related with six of these showing one to three band differences indicating a clonal origin.
Conclusion: There is evidence of co-carriage of VRE strains in MRSA patients thus setting the stage for possible emergence of vancomycin resistant *Staphylococcus aureus*. The presence of a single MRSA clone in patients and relatives indicates its transmission between the hospital and the community which poses a significant threat to public health.

P1448 High Panton-Valentine leukocidin prevalence among meticillin-sensitive *Staphylococcus aureus* from Yogyakarta, Indonesia

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Objectives: During the last decade, community-associated meticillin-resistant *Staphylococcus aureus* (CA-MRSA) has emerged. CA-MRSA is characterised by staphylococcal cassette chromosome mec (SCCmec) type IV or V, several continent-specific genetic backgrounds, and the presence of Panton-Valentine leukocidin (PVL). CA-MRSA clones could emerge through the transfer of the highly mobile SCCmec type IV element into PVL-positive meticillin-sensitive *S. aureus* (MSSA) with a genetic background common to CA-MRSA. Therefore, studies are needed to investigate the prevalence of PVL among MSSA isolates.
Methods: During September and October 2006, 440 nasal swabs were taken from outpatients (n = 320) and their companions (n = 320) at the Department of Ear, Nose and Throat at the Dr. Sardjito Academic Hospital in Yogyakarta, Indonesia. The swabs were send to The Netherlands for isolating and identification of *S. aureus*. These were characterised with spa typing and the algorithm based upon repeat pattern (BURP). The prevalence of the virulence factors collagen adhesion (CNA), PVL and toxic shock syndrome toxin 1 (TSST-1) was investigated.
Results: From 62 individuals (14%), including 39 outpatients (18%) and 23 companions (11%), *S. aureus* was isolated by standard microbiological methods. Typing revealed 37 spa types (of which 9 were new spa types), and 20 spa types were clustered into 6 spa-clonal complexes (spa-CCs) (42 isolates). The remaining isolates could not be classified into spa-CCs. Up to 69% of these isolates were associated with a genetic background common to the endemic MRSA clones, e.g. clonal complex (CC)1, CC8, and CC45. The remaining isolates were associated with CC12, CC72, and CC121. The prevalence of CNA, PVL and TSST-1 was 74%, 26% and 16% respectively; the presence of PVL was strongly associated with CC1.
Conclusions:
1. There was a low *S. aureus* prevalence (16%) among the population investigated, compared to the normal prevalence of between 30% and 50%.
2. No MRSA isolates were observed in the population.
3. A relatively high PVL-prevalence among MSSA isolates with a genetic background common to a virulent and epidemic CA-MRSA clone (CC1/USA400) was observed.
4. CA-MRSA clones might emerge through the integration of SCCmec type IV into PVL-positive MSSA with a genetic background common to CA-MRSA.
5. Studies are necessary to monitor the possible emergence of CA-MRSA clones, and limit their subsequent dissemination.

MRSA – a bit of a mixed bag

P1449 Community-acquired methicilin-resistant *Staphylococcus aureus* as a cause of pyonephrosis necessitating emergent nephrectomy. A new clinical entity

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Introduction: Community acquired-methicilin-resistant *Staphylococcus aureus* (CA-MRSA) carrying the Panton-Valentine leucocidin (PVL) toxin has been linked to skin and soft tissue infections and necrotising pneumonia. We report a new clinical syndrome related to this emerging pathogen.

Case presentation: An 84 year-old man, with history of diabetes mellitus type II, nephrolithiasis, recurrent urinary tract infections and prostatectomy, presented with constitutional symptoms and fever. He denied invasive procedures of the genitourinary tract in the last 2 years. Evaluation revealed tenderness of the right costophrenic angle, leukocytosis, CRP of 35.6 mg/dl and preserved renal function. A computed tomography revealed a right-sided staghorn calculus, with dilatation of the pelvicalyceal system and attenuation of renal cortex. Further focal infection was noted at the upper and middle pole of the right kidney along with enlargement of right ileopsoas muscle. Blood and urine cultures collected on admission were positive for MRSA. The patient's initial therapy with ticarcillin/clavulanate and amikacin was changed on the third hospital day to meropenem and vancomycin. The patient gradually became septic, necessitating right nephrectomy on the fourth hospital day. At surgery, the kidney was found small, with multiple scars and also with pus inside the renal pelvis and ureter. Perirenal tissue and psoas muscle were inflamed. The MRSA strain had the "community" phenotype with preserved sensitivity to trimethoprim-sulfamethoxazole and clindamycin). The assay for PVL toxin was positive, while PCR for mecA type III and IV was negative (PCR for mecA type V has been scheduled). Histopathology analysis reported chronic obstructive pyelonephritis secondary to nephrolithiasis, along with interstitial nephritis with tubular atrophy and nephrosclerotic glomerulae. Further infection was extended to pyelocalyceal system, ureter and peri-renal fatty tissue. The patient completed three weeks of intravenous vancomycin and was discharged in good condition, albeit with residual renal insufficiency.

Discussion: Community-acquired-MRSA differs from healthcare associated MRSA both in epidemiology and genetic characteristics. Recent reports indicate that CA-MRSA strains have entered tertiary care hospitals and are the cause of healthcare infections. This is a novel type of syndrome caused by CA-MRSA. Interestingly, the patient involved did have risk factors for invasive MRSA infection.

P1450 Family recurrent episodes of skin and soft-tissue infections by meticillin-susceptible *Staphylococcus aureus* carrying Panton-Valentine leukocidin

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Objectives: *Staphylococcus aureus* is a common pathogen able of causing a variety of infections in humans. Pathogenicity is due to several virulence factors, one of them is Panton-Valentine leukocidin (PVL). The aim of this study is to show the association between recurrent episodes of skin and soft-tissue infections in members of the same family, previously healthly, and the presence of meticillin-susceptible *S.aureus* (MSSA) carrying PVL.

Methods: Because of recurrent skin and soft-tissue infections in the members of the same family, nasal swabs were collected in all members from March to September 2007. Antimicrobial susceptibility of *S.aureus* isolated strains was performed by disk-diffusion method. Genotype characterisation of PVL was made by coamplification of the genes lukS-PV and lukF-PV by PCR. Genomic DNA was extracted from cultures grown on agar plates, by a modified DNAeasy tissue kit (QIAGEN) procedure. Genes were identified as a 433 pb band in the agarose gel after electrophoreses of amplified product.

Results: Recurrent skin and soft-tissue infections were diagnosed in 7 out of 8 members of the same family (father, mother, son #1, granddaughter, son #2, partner of son #2 and roommate of son #2; partner of son #1 was not affected) from April 2005 to September 2007, with no known exposure to healthcare establishments. Abscesses were located in armpit, back, thigh, knee, leg and buttocks. *S.aureus* was isolated in nasal swabs in 5 of the members; nasal swabs were negative in 2 members, and there were not data from the eighth. *S.aureus* was also recovered from one abscess in the armpit. All *S.aureus* isolates presented the same phenotype, resistance to penicillin, and susceptibility to oxacillin, clindamycin, gentamicin, levofloxacin, rifampin, TMP-SMX and vancomycin. The genes lukS-PV and lukF-PV were identified in all 5 of the *S.aureus* isolated in nasal swabs. All abscesses, except two, need surgical debridement. Treatment was made with amoxicillin-clavulanic. Because recurrence of abscesses despite the treatment and nasal decolonisation, patients carrying MSSA are being treated 5 days a month with nasal mupirocin and foamy chlorhexidine. At 6 months of follow up no recurrence has being reported.

Conclusions: Interfamily transmission of MSSA PVL-carrying strain could be the cause of the recurrent skin and soft-tissue infections. The decolonisation of nasal carriers of MSSA and hygienic measures could clear up the problem of recurrence of infections.

P1451 Stress! An investigation of emotional and information needs of three types of meticillin-resistant *Staphylococcus aureus*-carriers in the home situation

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Objectives: To analyse emotional and information needs of Meticillin Resistant *Staphylococcus aureus* (MRSA)-carriers in the home situation, in order to optimise patient education.

Methods: Semi-structured in-depth interviews were conducted with carriers of three different MRSA-types (n = 32): Community-Acquired (CA-MRSA; n = 3), Veterinary-Acquired (VA-MRSA; n = 3) and Hospital-Associated (HA-MRSA; n = 3), based on cognitive and emotional coping strategies. A focusgroup with representants of the three MRSA-types (n = 3) was performed to validate the interview results. Critical Incident Theory was used to detect stress incidents caused by MRSA-colonisation.

Results: MRSA-colonisation was particularly perceived as stressful by CA- and HA-MRSA carriers. Of the 826 reported stress events, 295 (36%) were mentioned by CA-MRSA carriers and 3 40 (41%) by HA-MRSA carriers. They experienced stress mainly because of feeling stigmatised and a lack of knowledge, leading to emotional problems and information needs (174 and 233 of 505 needs citations, respectively). Used strategies for emotional coping were seeking social support with family, friends, and colleagues. Information needs were met by actively searching for background and practical information.

VA-MRSA carriers reported relatively less stress events compared to CA- and HA-MRSA carriers (191of 826; 23%). Their coping strategies could be characterised by denial, suppression, and escapism.

In order to fulfil information needs, all carriers actively searched for information on the internet (51 of 164 citations on information resources), although the internet was perceived as an unreliable source providing ambiguous information. To fulfil emotional needs, CA-MRSA-carriers consulted the public health department, and HA-MRSA-carriers contacted the microbiologist, whose information was valued as insufficient to handle their complaints.

Conclusions: MRSA-colonisation turned out to be a stressful event for CA- and HA-MRSA-carriers, and therefore they had much more emotional and information needs compared to VA-MRSA carriers. Therefore, the provided patient education should depend on the type of MRSA. CA- and HA-MRSA-carriers have to be addressed more

personally, e.g., by a personal conversation with a healthcare provider so that emotional needs are met. VA-MRSA-carriers' needs can be met by providing them facts about MRSA.

P1452 First outbreak of MRSA ST398 in a Dutch hospital

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Background: In the Netherlands the rate of Meticillin resistant *Staphylococcus aureus* infections in hospitals is still low, but community acquired MRSA occurs more frequently. This increase is mainly caused by so called 'non-typable' MRSA (NT-MRSA, = not typable by PFGE using Sma1) belonging to MLST type ST398. This strain is widely disseminated among pigs, veal calves and people in contact with pigs. Transmission within families as well as solitary cases of colonised healthcare workers have been described but until now no nosocomial transmission to multiple patients or HCWs had occurred.

Outbreak: In June 2007 MRSA was isolated from a diabetic foot ulcer of a patient on a surgical ward. Subsequent screening of contacts among patients and healthcare workers revealed four additional patients with MRSA colonisation and/or infection and five HCW who carried MRSA. All strains were resistant to tetracycline and non-typable by PFGE. Spa typing showed that all strains to be t567, a type previously found in pigs. None of the patients had contact with pigs and/or veal calves. One healthcare worker lived on a pig farm but neither she nor her partner came into contact with pigs.

Conclusions: We describe the first outbreak of NT-MRSA among patients and HCW on a surgical ward. While the source is not fully established it could be the HCW living on a pig farm. This outbreak makes clear that transmission on a larger scale can occur, even with NT-MRSA.

P1453 Meticillin-resistant *Staphylococcus aureus* in horses and horse personnel at the Finnish veterinary teaching hospital, 2006–2007

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Objectives: We report the emergence of MRSA in horses at the Helsinki University Veterinary Teaching Hospital, in Finland during October 2006-June 2007.

Methods: After the first MRSA isolate in a horse was detected through routine wound infection surveillance in October 2006, active MRSA screening of horses at risk (hospitalised >24 h) was implemented. Swabs were obtained from nostrils, oral mucosa, perineum, and wounds. Voluntary screening of personnel (nostrils and skin lesions on hands) was performed on two occasions in 2006 and 2007. MRSA was confirmed by PCR of mecA and *S. aureus* specific nuc genes. MRSA isolates were characterised by pulsed-field gel electrophoresis (PFGE), spa typing, SCCmec and multilocus sequence typing (MLST). A case was defined as a horse or staff member with MRSA positive culture result. MRSA positive horses were handled with barrier nursing precautions in isolation or cohorts. The importance of hand hygiene and aseptic techniques in different procedures were emphasised.

Results: The first MRSA cluster with 2 wound infections and 3 colonisations among 98 horses (attack rate, AR 5%) occurred during October-December in 2006; all 24 screened personnel was negative. The 5 isolates were resistant to macrolides, fluoroquinolones and aminoglycosides, and identical in PFGE. MRSA strains were of ST125, spa type t1399, and SCCmec IVA. The second outbreak appeared in May 2007, and was also detected through wound infection surveillance. This cluster involved 3 infections and 10 colonisations among 61 horses (AR 21%). The index patient was a horse which was hospitalised during the first outbreak but was MRSA negative at that time. In staff screening, 1/25 person was colonised with MRSA. All 14 isolates were resistant to aminoglycosides and tetracyclines, but susceptible to fluoroquinolones and macrolides, were non-typeable by PFGE and possessed ST398, spa type t011, and SCCmec IV.

Conclusion: These are the first MRSA infections detected in horses in Finland. ST125 has been only seldom reported in humans in Finland, whilst ST398 is a new strain type in our country. Routine wound infection surveillance in the hospital was crucial in detecting MRSA. Early outbreak control measures, active screening of patients and staff training are necessary to prevent spread of MRSA in veterinary premises. Emerging MRSA infections in animals can cause a public health risk since strains causing infections in animals cause infections in humans and vice versa.

P1454 Prevalence of meticillin-resistant *Staphylococcus aureus* amongst residents and staff of nursing homes

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Objectives: It has been suggested that nursing homes can act as a reservoir for MRSA within the community and may contribute significantly to the spread of MRSA within hospitals when colonised residents are admitted for medical care. The aim of this study was, therefore, to determine the prevalence of MRSA amongst residents and staff in private nursing homes within the Northern Health and Social Care Trust (NHSCT) in Northern Ireland, and to compare strains isolated from nursing homes to those currently in circulation in local hospitals.

Methods: Swabs from the anterior nares were taken from 1,111 nursing home residents and 553 staff in 45 private nursing homes and processed by inoculation onto cefoxitin-containing chromogenic agar. After 48 h incubation, positive colonies were confirmed as MRSA by multiplex PCR using primers to detect staphylococcal 16S, nuc and mecA genes. MRSA strains were further analysed by restriction enzyme digestion (SmaI), followed by pulsed field gel electrophoresis (PFGE) and compared with strains isolated in local hospitals.

Results: The overall prevalence rate among residents was 23%, with individual home prevalences ranging from 0% to 73%. The overall prevalence among staff was found to be 7%, and again this ranged from 0% to 28%. Staff who were found to be colonised were employed in various positions throughout the nursing homes, ranging from care assistants to kitchen workers and maintenance. PFGE analysis showed that within a home, several MRSA strains could be present, but often, identical strains were shown to have colonised several individuals within one home. Staff were found to be colonised with the same strains as residents. No significant differences were determined by PFGE between nursing home and hospital MRSA isolates.

Conclusions: The results show that MRSA is prevalent within nursing homes in the NHSCT in Northern Ireland, although the extent of this prevalence may vary widely between nursing homes. Transmission of MRSA between nursing homes and hospitals is indicated by the identification of similar strains in both environments. Colonisation of staff was not limited to only those staff with a direct role in patient care and this possibly contributes to the transmission of MRSA within the nursing home environment. These results highlight the need for tailored infection control guidance for nursing homes.

P1455 The general public's beliefs about meticillin-resistant *Staphylococcus aureus*: a Mental Models approach

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Objectives: Meticillin Resistant *Staphylococcus aureus* (MRSA) is becoming an increasing public health threat. Risk communication strategies should create public awareness in order to prevent misconceptions leading to non-compliance with infection control measures. Therefore, risk communication should be matched to the general public's beliefs about MRSA. These beliefs were determined in this study.

Methods: The Mental Models Approach was applied. Based on the literature, a conceptual model was created which was used as an

interview scheme to elicit (in)correct public beliefs (n = 37). These beliefs served as items in a a close-ended questionnaire (n = 339) in order to estimate the prevalence of these beliefs among the general public.
Results: Although the majority of the general public (62%) had heard of MRSA via television (90%) and/ or newspaper (31%), great misconceptions exist. E.g., 26% of the public thought MRSA is a muscular disease, and 9% viewed MRSA as a synonym for repetitive strain injury.
After revealing the respondents that MRSA is also known as the hospital bacterium, they appeared to hold correct beliefs concerning risk factors and consequences of MRSA: 72% believed that MRSA may cause infections, 59% was convinced hospitalised MRSA-carriers are treated in isolation, that a weakened immune system increases one's risk of getting MRSA-infections (75%), and 62% was aware that MRSA can also be found outside the hospital. Respondents were less aware about prevention, reservoir, treatment, and transmission of MRSA: 70% incorrectly assumed vaccination would prevent MRSA-colonisation. While 39% thought MRSA can be found in the blood, only 16% assumed MRSA lives on the skin, and 64% of the sample was unaware that most antibiotics are ineffective against MRSA. 7% supposed MRSA is transmitted by insects, and 56% did not know whether MRSA could be found at cattle or not.
47% of the respondents believed that MRSA is a serious risk for society, although MRSA was not experienced as a personal health threat.
Conclusions: Although the Dutch general public recognises the well-known risk factors and consequences of MRSA and is slightly aware of its threat to society, many misconceptions exist. Important facts, like MRSA-prevalence among cattle and the presence of MRSA on the skin, are unfamiliar to the public. Risk communication should show attention to these misconceptions in order to raise public awareness and prevent non-compliance with control measures.

P1456 Public knowledge and perceptions of MRSA: results of the Tayside Survey

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Objective: To establish levels of knowledge and concern about MRSA in the general public in Tayside, Scotland.
Methods: This opportunistic anonymous questionnaire survey was carried out in 15 general practice surgery waiting areas and two other establishments. Data collected included respondent demographics, whether and where respondents had heard of MRSA, knowledge of possible modes of transmission, treatment possibilities, level of personal concern, and the perceived accuracy of media information.
Results: Questionnaires were completed by 1000 adults (>16 years old). 59% of respondents were female. 802 (80%) were white Scottish with 4% from non-UK ethnic groups. The age range and ethnic distribution of respondents were representative of the Tayside population.
856 (86%) of those surveyed had heard of MRSA, 66% via the media. 591 (59%) knew it is a bacterium rather than a virus. The possibility of healthy carriage was known about by 467 (47%) of respondents, and 32% knew someone who had had MRSA.
Respondents blamed lack of staff hand washing (73%) and lack of hospital cleanliness (68%) for MRSA infection in hospitals. 50% thought a patient with MRSA infection could have been infected by an asymptomatic visitor, while 36% knew that infection could come from patients' own carriage. 47% of respondents knew that antibiotics could be used to treat MRSA.
32% of those surveyed were "a little worried" that if admitted to hospital they might get MRSA, 20% were "very worried" and 0.7% were "not at all worried". Only 59% of respondents stated their perceived level of risk of getting MRSA infection if admitted to hospital. Answers ranged from <1% (n = 37) to 75+% (n = 33) with most people selecting between 10% and 50%.
Respondents doubted the accuracy of information about MRSA in newspapers with 27% thinking it is inaccurate and 36% "not sure". 55% wanted more information about MRSA citing GP waiting areas as suitable locations.
Conclusions: This large survey has given new insight into the knowledge and perception of MRSA among the general public. This knowledge is reasonably good in some areas but lacking in others. The general public are sceptical of media reporting and want more information about MRSA.

P1457 First case of MRSA spondylodiscitis treated successfully with linezolid

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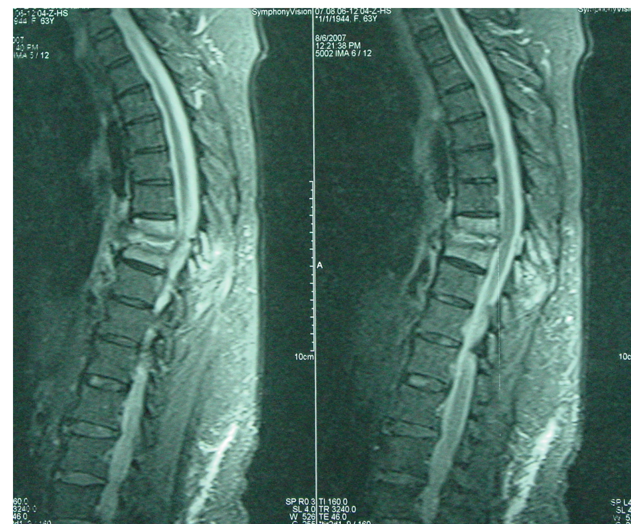
Introduction: Vertebral osteomyelitis is primarily a disease of adults, with the majority of patients being more than 50 years old. Hematogenous spread from a distant site or focus of infection has remained the main route for pathogenesis of vertebral osteomyelitis. We present an MRSA-induced pyogenic spondylodiscitis in an immunocompetent host, treated successfully with linezolid alone, which occurred 4 months following a nosocomial MRSA decubitus ulcer infection.
Case Report: A 50-yr-old woman was admitted for severe back pain and low grade fever of 1.5 months duration. Her back pain had started after she had been discharged from a hospital where she had stayed for 4 months for cerebrovascular accident. Her medical history records also revealed that during her hospitalisation she had multiple episodes of decubitus ulcer infections with MRSA. Physical examination showed no remnants from stroke but limited activity due to severe back pain upon movement, including getting out of bed or even turning over in bed. Her severe pain had markedly restricted ambulation and other activities of daily living. She did not show any neurological deficits.
Thoracolumber Magnetic-Resonance Imaging (MRI) was characteristic of T8–T9 spondylodiscitis with loss of height of both vertebral corpi, vertebral body and disk space enhancements and paravertebral soft tissue involvement with abscess formation. Fine needle aspiration of this region yielded Gram-positive cocci identified as Meticillin resistant *S. aureus*.
She was started linezolid that she received for 12 weeks (600 mg iv, every 12 hours for 6 weeks and 600 mg po, every 12 hours for 6 weeks) and by the third week of therapy, she showed considerable improvement, her CRP level was normalised, need for analgesia disappeared and was able to move in bed and walk. The patient was monitored for adverse drug events but none occurred. She was discharged following an uneventful treatment course and is doing well.
Discussion: Our case is the first case of an MRSA spondylodiscitis, reported in English language literature, treated successfully with linezolid. We neither encountered any complications nor any surgical intervention was necessary. Controlled trials are necessary to show the efficacy or superiority, if any, of linezolid to glycopepetides in the treatment of MRSA spondylodiscitis following such promising case reports with satisfactory outcomes.



P1458 **Comparison of mortality associated with bacteraemia caused by meticillin-resistant *Staphylococcus aureus* on arrival at hospital versus hospital-acquired MRSA bacteraemia: a cohort analysis from the BURDEN study group**

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Objective: MRSA bacteraemia is generally regarded as a hospital acquired infection so may be less likely to be treated empirically when present on arrival at hospital. We therefore tested the hypothesis that mortality associated with MRSA bacteraemia on arrival at hospital is greater than mortality with hospital acquired MRSA bacteraemia.
Methods: The study population was adults (age 18 or over in 2005) resident in Tayside, Scotland from 1st January 2005 to 30th September 2006 who had a new admission to Ninewells Hospital between 1st July 2005 and 30th June 2006. The cohort enrolled patients from this population who were admitted to wards where at least one case of bacteraemia with MRSA occurred in the study period. We defined bacteraemia on arrival at hospital as a positive blood culture within 48 h of admission and hospital acquired bacteraemia as a positive blood culture ⩽48 h after admission. The comparator group was all patients admitted to the same wards in the same time periods without *S. aureus* bacteraemia. The outcome was 30 day mortality, ascertained from the national registry of deaths. We used multivariable analysis to estimate the relative risk of mortality associated with MRSA bacteraemia adjusted for age, sex and Charlson co-morbidity index (CCI) calculated from the discharge diagnoses of the index admission and of any hospital admission in the previous year.
Results: The cohort included 4,397 patients; 21 had MRSA bacteraemia on arrival, 34 had hospital acquired MRSA bacteraemia and 56 had MSSA bacteraemia. In comparison with other cohort patients those with MRSA bacteraemia were older (mean age 67 vs 63) and had more comorbidities. Mean CCI was 3.76 for patients with MRSA bacteraemia on arrival, 2.56 for hospital acquired MRSA bacteraemia patients and 1.91 for the comparator patients. In comparison with patients with a CCI of 0 the relative risk of 30 day mortality was 1.98 with CCI 1–2, 2.65 with CCI 3–5 and 4.89 with CCI 6 or greater. After adjustment for age, sex and CCI the relative risk of 30 day mortality with MRSA bacteraemia on arrival at hospital (2.48, 95% CI 0.84–7.27) was higher than with hospital acquired MRSA bacteraemia (1.11, 95% CI 0.38–3.29) and also higher than with MSSA bacteraemia on arrival at hospital (1.29, 95% CI 0.38–4.39).
Conclusions: Our cohort study supports the hypothesis that MRSA bacteraemia on arrival at hospital is associated with a higher 30 day mortality than hospital acquired MRSA bacteraemia.

P1459 **Characterisation of baseline meticillin-resistant *Staphylococcus aureus* isolates from a phase IV clinical trial of complicated skin and soft tissue infections**

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Objective: To characterise meticillin-resistant *S. aureus* (MRSA) associated with complicated skin and soft tissue infections (cSSTI) collected during a Phase IV clinical trial comparing linezolid with vancomycin for the treatment of cSSTI due to MRSA.
Methods: 532 MRSA baseline isolates were collected from subjects with cSSTI in Latin America (LA; 5 countries, 68 isolates), Europe (EU; 6 countries, 112), Asia (Singapore [1] and Malaysia [2]), South Africa (13) and the United States (USA; 336). Susceptibility testing was performed by CLSI broth microdilution method. Isolates were screened for heterogeneous resistance to vancomycin (hVISA) using the macro Etest method. The presence of inducible clindamycin (CC) resistance (R) phenotype was assessed by D-test; PVL genes and SCCmec types by PCR and clonality was evaluated by PFGE. Dominant PFGE type strains from each country were further evaluated by spa typing and multilocus sequencing typing (MLST).
Results: Most active antimicrobial agents were: linezolid = glycopeptides (teicoplanin and vancomycin, 0% R) < quinupristin/dalfopristin (0.2% R) < trimethoprim/sulfamethoxazole (4.7% R) < tetracycline (12.6% R) < CC (45.9% R; 18.6% inducible R plus 27.3% constitutive R) < gatifloxacin (67.1%). (Table). CC-R rates were highest in Asia and South Africa (100.0%), followed by LA (86.8%), EUR (78.6%) and the USA (24.1%). Most isolates were R to erythromycin (ERY; 92.9%) and 29.2% of ERY-R, CC susceptible (S) isolates had a positive D-test (inducible CC-R). Five (0.9%) isolates were characterised as hVISA, 4 from EUR and 1 from LA. 278 (52.3%) isolates were PVL-positive and 96.8% of those were from the USA. The remaining PVL-positive isolates were from Colombia (4) and Venezuela (5). 373 (70.1%) isolates were SCCmec type IV, 89 (16.7%) type II, 44 (8.3%) type I and 26 (4.9%) type III. Isolates from the USA showed SCCmec type IV (81.2%) or II (18.8%). The majority of USA isolates clustered within the USA300 PFGE type (77.1%) or USA100 (15.8%). Most countries had a dominant and unique clone.

Table

Antimicrobial agent	MIC_{50}	MIC_{90}	% S	% R
1. Linezolid	2	4	100.0	–[a]
2. Erythromycin	>16	>16	7.1	92.1
3. Clindamycin	0.25	>4	54.1	45.9[b]
4. Gatifloxacin	2	>8	32.2	67.1
5. Tetracycline	0.25	>16	86.8	12.6
6. Trimethoprim/sulfamethoxazole	⩽0.5	⩽0.5	95.2	4.7
7. Quinupristin/dalfopristin	0.25	0.5	99.8	0.2
8. Teicoplanin	1	1	100.0	0.0
9. Vancomycin	1	1	100.0	0.0

[a]No resistance breakpoint has been established by the CLSI.
[b]Includes constitutive and inducible resistance phenotypes.

Conclusions: All MRSA isolates were S to linezolid and glycopeptides, and resistance rates were high for ERY, CC and gatifloxacin. The prevalence of hVISA was low overall and this phenotype was not observed in the USA. MRSA in the USA were SCCmec IV and PVL positive, previously associated with community acquired-MRSA; while in the rest of the world the distribution of SCCmec types and PVL genes varied by geographical region.

Reduced vancomycin resistance in *S. aureus*

P1460 **Influence of the method for detecting reduced vancomycin susceptibility in meticillin-resistant *Staphylococcus aureus* bacteraemia. A 14-year study**

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Objectives: Actually, in clinical cases of methicilin-resistant *Staphylococcus aureus* infections with low vancomycin susceptibility (MIC > 1 mg/L) (MRSA-LVS) the new drugs are indicated like alternative treatment. The aims of this study were to kwon the prevalence of MRSA-LVS strains in bloodstream infections in our hospital and the influence of the method used in routine diagnosis for detecting reduced vancomycin susceptibility.
Methods: From 1994 to november-2007, 172 patients with a clinically significant MRSA nosocomial or community-adquired bacteraemia were detected in the Microbiology Department of a university hospital. MIC vancomycin determination was performed by automated (VITEK 2 – bioMérieux) and macro E-Test (AB Biodisk) methods. A compared study of the MIC vancomycin obtained by both methods in each year of the period was analised.
Results: During the last 14 years, twenty-one (12.2%) of the MRSA bacteremic infections showed a LVS (MIC > 1 mg/L) with the VITEK-2

results, in front of 84.3% MRSA-LSV (145 cases) detected by E-Test method. MIC of all strains was <4 mg/L with VITEK 2. Fifteen of them (8.7%) showed a MIC ≤4 mg/L by E-Test. The analysis by VITEK 2 from different periods of the study display a significant progressive increase of the MRSA-LVS percentage (1994–99: 6.1%; 2000–03: 7.3%; 2004–2005: 12.8%; 2006: 14.8%; and 2007: 25.1%). However, by E-Test the obtained MIC were usually higher since in the 1994–2000 period all MRSA-LVS were MIC 2 mg/L (54.5%) and in 2000–07 period more than 90% showed a MIC ≤ 2 mg/L. No vancomycin resistant was detected.
Conclusion: Detection in laboratory routine of MRSA with reduced vancomycin susceptibility is depending on the microbiology method used. The automated systems show a progressive increase of the MRSA-LVS bacteremic cases, but is not correlated whit the E-Test MIC results. The method used to detect MRSA-LVS and their prevalence must be interpreted cautiously.

P1461 Relationship between hVISA and outcomes among patients with MRSA bloodstream infections treated with vancomycin

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Objectives: To examine the relationship between MRSA strains express heterogenous resistance to vancomycin (hVISA) and outcomes among patients with MRSA bloodstream infections treated with vancomycin.
Methods: Study period: 1/05–4/07. Inclusion criteria: (1) ≤18 yrs, (2) MRSA bacteraemia (met CDC criteria), (3) received vancomycin therapy within 48 hours of index MRSA blood culture, and (4) survived >24 hours after start of therapy. Demographics, co-morbid conditions, microbiology, and treatment data were collected. MRSA strains with an MIC value ≥6 mg/L on high inoculum brain heart infusion agar plates were considered hVISA (method of Walsh et al). Failure was defined as in-hospital mortality, bacteraemia ≤7 days on vancomycin therapy, or recurrence of MRSA bacteraemia within 60 days of vancomycin discontinuation. Length of stay (LOS) after start of vancomycin was also compared between groups. Parameters associated with failure by bivariate analysis ($P < 0.2$) were included a multivariate analysis and a stepwise approach was used to identify independent predictors.
Results: During the study period, 92 patients met the inclusion criteria. Of the 92 patients, 17 (18.5%) were hVISA positive. Comparison of outcomes between hVISA and non-hVISA patient groups are provided in the table. In the multivariate analysis, the parameters independently associated with failure were hVISA (Adjusted odds ratio (AOR), 4.3; 95% CI: 1.3–14.5, p-value=0.02), APACHE-II score ≤22 (AOR, 16.9; 95% CI: 1.9–80.2, p-value=0.01), and dialysis (AOR, 3.9; 95% CI: 1.3–11.8, p-value= 0.02.

Bivariate analysis of outcomes between hVISA patient groups

Outcomes	hVISA (n = 17)	Non-hVISA (n = 75)	p-value
Overall failure	11 (64.7)	21 (28.0)	0.004
In-hospital mortality	4 (23.5)	13 (17.3)	0.5
Bacteraemia ≥7 days	6 (35.3)	5 (6.7)	0.004
Recurrence within 60 days	6 (35.3)	6 (8.0)	0.003
Hospital LOS after index culture collection, median days (IQR)	20 (13–32)	13 (7–36)	0.3

*All data presented as number (percent) unless otherwise noted.

Conclusions: The data strongly suggest that patients with hVISA MRSA bloodstream infections respond poorly to vancomycin. Alternative anti-MRSA therapies should be considered for these patients.

P1462 Predictors of hVISA among patients with MRSA bacteraemia

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Objectives: The objectives were (1) to determine the proportion of MRSA bloodstream infections at our institution that express heterogenous resistance to vancomycin (hVISA) and (2) to identify clinical predictors of hVISA among the patient population.
Methods: A cross-sectional study was conducted at Albany Medical Center (Albany, NY). All patients with a positive MRSA bloodstream culture between 01/2005 and 5/2007 were eligible. Inclusion criteria: age ≤18 years, non-neutropenic, and MRSA blood culture met CDC criteria for infection. Demographics, co-morbid conditions, microbiology, and antibiotic exposure data were collected. MRSA strains with an MIC value ≤6 mg/L on high inoculum brain heart infusion agar plates were considered hVISA (method of Walsh et al). Parameters associated with hVISA by bivariate analysis ($P < 0.2$) were included a multivariate analysis and a stepwise approach was used to identify independent predictors. The CART technique was used to identify significant breakpoints in continuous clinical features that were associated with an increased proportion of hVISA.
Results: During the study period, 103 patients the inclusion criteria. Of the 103 patients, 20 (19.4%) were hVISA positive. Bivariate comparison of clinical paramaters between patients with hVISA and non-hVISA are provided in the table. Source of infection did not differ between groups ($P = 0.5$). In the logistic regression, recent history of healthcare institution exposure (AOR= 5.7, 95% CI: 1.2–27.8, $p = 0.03$) and creatinine clearance (Crcl) ≤27.6 ml/min (AOR=5.3, 95% CI: 1.8–15.6, $P = 0.002$) were the only independent predictors of hVISA.

Bivariate analysis of clinical features between hVISA patient groups

Clinical feature	Non-hVISA	hVISA	p-value
Age >59 years*	43 (51.8)	17 (85.0)	0.007
Weight in kg, mean (SD)	82.2 (24.5)	85.5 (24.3)	0.6
Creatinine clearance <27.6 ml/min*	19 (22.9)	12 (60.0)	0.001
Recent healthcare institution exposure	52 (62.7)	18 (90.0)	0.02
Dialysis	19 (22.9)	7 (35.0)	0.3
Diabetes mellitus	34 (41.0)	13 (65.0)	0.05
Heart failure	26 (31.3)	5 (25.0)	0.6
COPD	21 (25.3)	4 (20.0)	0.6
Recent surgery in prior 30 days	18 (21.7)	9 (45.0)	0.03
Recent antibiotics in previous 30 days	51 (61.4)	9 (45.0)	0.2
Prior vancomycin	15 (18.1)	1 (5.0)	0.2
Length of stay prior, median (IQR)	1 (0–13)	0.5 (0–12)	0.8
Intensive care unit at onset	23 (27.7)	7 (35.0)	0.5

All data presented as number (percent) unless otherwise indicated.
*CART-derived breakpoint.

Conclusions: Recent healthcare institution exposure and CrCl ≤27 mL/min were highly predictive of hVISA. This knowledge can be used to direct therapy decisions by targeting aggressive therapy in pts at highest risk for hVISA.

P1463 Heteroresistant vancomycin-intermediate *Staphylococcus aureus* is associated to recurrent bacteraemia with the same strain

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Objectives: Recurrence of bacteraemia caused by *Staphylococcus aureus* appears despite administration of antibiotics active in vitro and can lead to increase in patient morbidity and mortality. The aim of study was to report the prevalence rate of recurrent bacteraemia due to *S. aureus* in our institution and to define the clinical and epidemiologic characteristics of relapse [isolates with the same Pulsed Field Gel Electrophoresis (PFGE) pattern] and reinfection (different PFGE pattern). Furthermore,

to investigate whether relapse with *S. aureus* meticillin-resistant (MRSA) was associated with heteroresistance to vancomycin.

Patients and Methods: A retrospective study was conducted between Jan 2001 and Dec 2006. It included all patients who presented at least two episodes of *S. aureus* bacteraemia and recovered after a full course of antibiotic therapy of the first clinical episode. All isolates underwent a PFGE. A Etest macromethod (BHI agar with an inoculum of 2McF) was performed on the isolates of the first episode of MRSA bacteraemia and on the isolates of the control group of patients with no recurrence. MIC$\leqslant$8 mg/L defined heteroresistance to vancomycin.

Results: During the study period, 883 patients had at least one episode of *S. aureus* bacteraemia. Forty three patients had a recurrence with 90 episodes. The recurrence rate was 5%. The PFGE method indicated that of the 45 episodes of recurrence, 34 (75.5%) were relapse and 11 (24.5%) reinfection. All reinfections were caused by *S. aureus* meticillin-susceptible (MSSA) whereas a 58.8% of relapses were caused by MRSA. According to the univariate analysis, MRSA bacteraemia was a risk factor for relapse ($p < 0.001$). Comparing MRSA relapses with the control group, we found that diabetes, haemodialysis and Etest macromethod with MIC$\leqslant$8 mg/L to vancomycin, were associated with an increased risk of recurrence. From the multivariate logistic regression, only the Etest macromethod with a MIC $\geqslant$ 8 mg/L to vancomycin was a predictor of recurrence (OR=4.4, CI 95% (1.03–19.3) $p < 0.044$.

Conclusions: MRSA is primarily associated with relapse whereas MSSA is more associated with reinfection. Diabetes and haemodialysis are risk factors for recurrent bacteraemia. Although we think that recurrent bacteraemia occurs as a result of multiple factors, heteroresistance to vancomycin seems to be a probable predictor of relapse. A result of MIC$\leqslant$8 mg/L to vancomycin could keep us alarmed of heteroresistance and furthermore of a possible relapse.

Clostridium difficile: epidemiology

P1464 First cluster of clindamycin-resistant *Clostridium difficile* PCR-ribotype 027 associated disease in Switzerland

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Objectives: Since June 2006, all patients with *Clostridium difficile*-associated diarrhoea (CDAD) are cultured for the presence of *C. difficile* at University Hospital Basel (UHBS) and *C. difficile* isolates are characterised regarding toxin profile (toxin A/B, binary toxin A/B), PCR-ribotype, genotype of the putative negative regulator gene (tcdC) and antibiotic susceptibility. In the time period between October 2006 and May 2007 a cluster of *C. difficile* PCR-ribotype 027 at UHBS and a geriatric hospital was detected and further investigations were performed.

Methods: Genes for toxins A/B and binary toxins were detected by PCR; tcdC was amplified and sequenced. PCR-ribotyping and multiple-locus variable-number tandem-repeat analysis (MLVA) were performed using standard protocols. Clinical information was obtained by standardised questionnaires.

Results: From October 2006 to May 2007, the incidence of CDAD at the geriatric hospital increased from zero to 0.8 cases per 1000 patient days in March, whereas the incidence at UHBS has not changed. A total of 16 patients with CDAD caused by *C. difficile* strain PCR-ribotype 027 was detected in the time period. The index case was identified in October 2006 as a 82-year old female patient previously hospitalised abroad. *C. difficile* ribotype 027 was detected in two other patients at UHBS, whereas 13 cases were detected at the geriatric hospital. The outbreak ended by the end of June 2007 and since then no other case with this strain was observed despite intensive epidemiological and laboratory-based surveillance. The median age of the 16 patients was 83.5 years (interquartile range 79–92). All isolates were highly resistant to moxifloxacin (MIC > 32 mg/L) and clindamcyin (MIC > 256 mg/L). Genes for toxins A/B, binary toxins and ermB were detected by PCR in all strains. tcdC gene showed a 18-bp deletion and a single nucleotide deletion at position 117. MLVA typing revealed one cluster of genetically highly related (STRD < 2) clindamcyin-resistant PCR-ribotype 027 strains which differed considerable from control 027 isolates.

Conclusions: We report the first cluster of clindamycin-resistant *Clostridium difficile* PCR-ribotype 027 in Switzerland, most likely imported from abroad. When the index patient was transferred to a geriatric hospital the strain did propagate, but remained undetected by routine epidemiological surveillance until molecular characterisation of strains was performed. MLVA proved to be a valuable new typing tool.

P1465 A two-centre study to determine possible geographic differences in the prevalence of *Clostridium difficile* ribotypes in Scotland

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A mandatory national surveillance programme for *Clostridium difficile* was introduced in Scotland in 2006 in response to its increasing prevalence among nosocomial infections in the UK and other countries. In a parallel prospective typing study, ribotype 106 accounted for more than 65% of isolates examined in Scotland while the 027 hypervirulent strain was detected only twice. These findings vary significantly from reports from England and Wales where ribotypes 001, 027 and 106 each accounted for approximately 25%, and an earlier study in south east Scotland where type 001 accounted for more than 75%. This suggests possible geographical or temporal differences in the prevalence of circulating types.

Objectives: This study was undertaken to determine the ribotype prevalence of *C. difficile* in two large teaching hospitals in two distinct geographical regions of Scotland, one in the west and one in the east, over the same period in May 2007–June 2007.

Methods: Faecal samples were obtained from consecutive, toxin-positive patients (50 from hospital E (east) and 54 from hospital W (west)). *C. difficile* was cultured from 97 samples and these isolates were typed by PCR-ribotyping according the method described by O'Neill et al. (1996). Ribotype patterns were compared with reference strains obtained from the Anaerobe Reference Laboratory, Cardiff. Patterns were analysed and compared using BioNumerics® software.

Results: Ribotype 106 was the predominant type in both hospital E (62.2%) and hospital W (65.4%). Type 001 accounted for 13.3%(E) and 11.5%(W), type 002 accounted for 4.4%(E) and 5.8%(W), type 005 accounted for 2.2%(E) and 3.8%(W). Single isolates of types 018 and 020 were found in both hospitals E and W. Two isolates of 015 and a single isolate of 026 were found only in hospital E while single isolates of 012, 014, 017 and 081 were isolated from hospital W. The patterns from four isolates produced previously unidentified ribotype patterns that have yet to be matched with patterns in the UK database.

Conclusion: The prevalence of the most common PCR-ribotypes of *C. difficile* isolated at two hospitals during the same time period were the same suggesting no geographic difference exists in Scotland. This result supports data from the prospective study indicating that differences exist in the distribution of ribotypes observed in Scotland and in England & Wales. Furthermore, type 106 has now replaced type 001 as the predominant type in Scotland.

P1466 Community-diagnosed *Clostridium difficile* infection: preliminary results from enhanced surveillance

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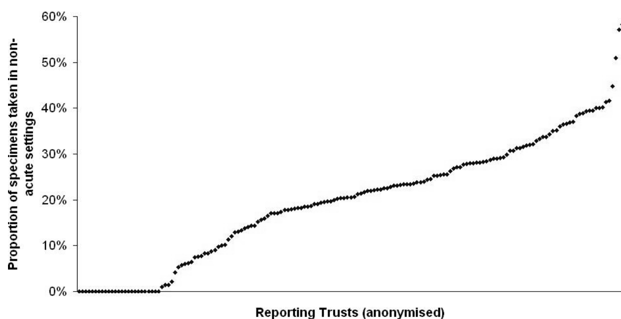
Objectives: To assess the contribution of cases presenting in the community to the burden of *Clostridium difficile* infection as reported through the English mandatory surveillance system.

Methods: Since April 2007, enhanced surveillance data on *C. difficile* infections have been collected through a web-enabled reporting system. All NHS (National Health Service) acute Trusts (hospital groups) in England are required to report all *C. difficile* positive specimens meeting the case definition which are processed by their laboratories. The dataset

includes a field indicating the patient location (e.g. acute trust hospital, GP) at the time of specimen collection. This analysis refers to records with specimen dates in July – September 2007.

Results: ~13,000 cases of *C. difficile* infection were reported through the web-based system between July and September 2007. Overall, 22% of records gave the patient location at the time of specimen collection as being outside an acute Trust hospital. This was highly variable between Trusts, ranging from 0% to 58% (Figure). 16% of specimens reported by Acute Teaching Trusts were obtained in non-acute settings; in Large Acute, Medium Acute and Small Acute Trusts the figure was ~22–25%. In specialist Trusts, all *C. difficile* positive specimens were taken from patients in acute Trust hospitals.



Proportion of *C. difficile* positive specimens taken in non-acute settings: England, July–September 2007.

Conclusion: A substantial proportion of *C. difficile* positive specimens processed by acute Trust laboratories, and reported as per the requirements of the mandatory surveillance scheme, are taken in non-acute settings. Planned refinements to the surveillance system will help to better identify what proportion of these infections, as well as those detected within the acute setting, are community-acquired. These findings will inform the definition of the Department of Health's planned *C. difficile* target.

P1467 Phenotypic and genotypic characterisation of *Clostridium difficile* isolates in French hospitals from 2001 to 2007

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Clostridium difficile is an important cause of nosocomial bacterial diarrhoea in adults. Recent outbreaks of *C. difficile*-associated disease (CDAD) have been described in North America and Europe related to the emergence of a hypervirulent *C. difficile* strain NAP1/027 that is resistant to erythromycin and moxifloxacin.

Objectives: The objective of this study was to follow the epidemiology of CDAD in Jean Verdier-René Muret hospitals over a 6-year period by investigating antibiotic susceptibility and molecular characteristics of *C. difficile* isolates.

Methods: Fifty strains isolated between 2001 and 2007 from patients with CDAD were selected. Toxins A and B were detected with an immunoenzymatic method (ICTAB, Meridian). Binary toxin was detected by cdtA and cdtB PCR according to Stubbs et al. Antibiotic susceptibility to metronidazole, vancomycin, erythromycin, moxifloxacin and linezolid was determined by the disk diffusion method. MICs of metronidazole, vancomycin and tigecyclin were determined by the E-test method. Isolates were typed by PCR-ribotyping according to Bidet et al.

Results: From 2001 to 2007, all the isolates studied were susceptible to metronidazole, vancomycin and tigecyclin. In contrast, we observed a significant decrease of susceptibility to moxifloxacin (100% in 2001 vs 28.5% in 2007) and to erythromycin (60% in 2001 vs 14% in 2007). Toxins A/B were detected in all the isolates. The binary toxin was detected in 15% of the isolates; its presence was not correlated with a specific PCR-ribotype. Toxin profile TcdA+ TcdB+ CDT− was the most common (81.8% of isolates). PCR-Ribotype "18" was the most prevalent detected since 2006. The isolates displaying this PCR-ribotype were resistant to erythromycin and moxifloxacin and were principally found in the same unit, suggesting cross infection.

Conclusion: This study showed that diverse clones of *C. difficile* circulated during these 6 years with stable susceptibility to metronidazole and vancomycin. Recently, an epidemic strain ribotype "18" resistant to erythromycin and moxifloxacin has emerged in one unit where fluoroquinolones are frequently used demonstrating the role of antibiotic selection pressure. The emergence of this strain could explain the significant decrease of susceptibility to moxifloxacin and erythromycin observed in 2007. However, today, no isolate with a ribotype matching PCR-ribotype 027 was detected.

P1468 Epidemiology of *Clostridium difficile* in Scotland – A possible shift in ribotype distribution

D.J. Brown, C. Wiuff, H. Mather, J.E. Coia (Glasgow, UK)

Clostridium difficile Associated Disease (CDAD) is considered the leading cause of healthcare associated diarrhoea in Scotland. Surveillance of CDAD became mandatory in Scotland in 2006 in persons over 65 years old in the healthcare setting. However, only few isolates have been typed under the mandatory programme in its first year. At the same time a prospective ribotyping study was carried out by an enteric pathogen laboratory. The results of this study, which is the most recent molecular typing study of *C. difficile* isolates from across Scotland, are presented here.

Objective: The aim of this study is to determine the current distribution of ribotypes of *C. difficile* obtained from hospital patients across Scotland, and by comparison with previous typing studies determine whether a shift in the strain distribution of *C. difficile* has occurred.

Methods: 130 isolates were submitted to the laboratory over a period of 18 months from 12 hospitals in 4 healthboard areas in Scotland. All isolates were typed using PCR ribotyping according the method described by O'Neill et al. (1996). Variable-length intragenic spacer regions of the rRNA complex were amplified by PCR, and ribotype patterns were compared directly with those of reference strains obtained from the UK national reference laboratory for *C. difficile* in Cardiff.

Results: Most isolates were ribotype 106 (64.6%), with 001 (23.1%), 077 (3.1%), 015 (1.5%) and two isolates of the hypervirulent ribotype 027 (1.5%) were also identified as part of this study. Furthermore, single isolates of ribotypes 002, 005, 078, 081 and 188 were identified. A total of 3 isolates produced previously unidentified ribotype patterns, which have yet to be matched with patterns in the UK database. The less common ribotypes and the unidentified ribotypes amounted to 6% of all typed isolates. The distribution of ribotypes was stable over the18-month period.

Conclusion: Recent data from England and Wales have indicated that ribotypes 106, 027 and 001 each account for about 26% of cases in 2005. A previous study carried out in south-east Scotland in 2005 identified ribotype 001 in 75.8% of cases. This current study demonstrates that ribotype 106 has become the predominant ribotype in Scotland. Ribotype 027 was also isolated for the first time in Scotland in this study.

P1469 The epidemiology of the second phase of a hospital outbreak of *Clostridium difficile*-associated diarrhoea, Stoke Mandeville, UK

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Objectives: To establish the epidemiology of the second phase of a hospital outbreak of *Clostridium difficile* associated diarrhoea (CDAD outbreak at Stoke Mandeville Hospital (SMH) from 1 December 2004 to 31 May 2005, the cases due to ribotype 027, risk factors and document control measures.

Methods: 229 incident cases of CDAD and readmissions with positive isolates for both admissions to Buckinghamshire Hospitals NHS Trust were included. Those with onset of symptoms more than 3 days from admission were considered hospital acquired cases and those

with symptom onset within 72 hours of admission were considered community acquired. Relapses were defined as 2 confirmed diagnoses more than 3 months apart. Patient demographic and clinical information was extracted from the case notes in compliance with data protection and patient confidentiality requirements as agreed by the Local Research Ethics Committee. Autopsy findings were extracted from pathology systems and infection control measures obtained from the infection control records and clinical notes. Data analysis used STATA 8.2.
Results: The incidence of CDAD at SMH was 2.11/1000 bed days and accounted 5312 bed days. The monthly incidence was highest in the winter months with a peak in January. Most cases were due to the 027 ribotype. Eighty eight percent of patients were over 65 years of age, 60% were female and 25% were community acquired. The mean length of stay (LOS) in hospital before onset of symptoms was 12.4 days, with an additional mean LOS of 35.4 days before discharge. MRSA coinfection occureed in 16.3%. The outbreak was associated with high levels of ciprofloxacin use. CDAD attributable mortality was 15%.
Conclusion: This study confirmed 027 ribotype as an outbreak strain in the UK with high mortality and morbidity rates which raised the national profile of CDAD. Applying the national additional hospital cost of £4000/CDAD the outbreak cost its NHS Trust about £900,000 over 6 months.

P1470 Costs of nosocomial *Clostridium difficile*-associated diarrhoea

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Objectives: Nosocomial *Clostridium difficile*-associated diarrhoea (CDAD) is a common infection in hospitals. A matched case-control study was done to determine hospital-wide excess costs due to CDAD.
Methods: Cases of CDAD were assessed by prospective hospital-wide surveillance in a tertiary care university hospital in 2006. Nosocomial CDAD cases (>72 hours after admission) were matched to control patients without CDAD in a ratio 1:3 using: (a) the same DRG in the same year, (b) a control's length of stay (LOS) $\leqslant$ time frame of cases before infection, (c) a Charlson co-morbidity index ± 1. Data on overall costs per case were provided from the financial controlling department. We excluded patients in psychiatry (who do not receive DRG codes) and paediatric patients. Evaluation of the application of matching criteria was done by Wilcoxon test for independent samples and Fisher's Exact test. Differences in LOS and costs between matched cases and controls were calculated following three parameters for the matched pairs: (a) difference in the LOS, (b) difference in costs per patient, (c) difference in costs per patient-day. For all parameters median with 95% confidence interval (CI95) non-parametric (distribution free) were calculated. A p-value <0.05 or a CI95 that excludes the zero were considered significant.
Results: There were a total of 116 CDAD cases in 2006. Data on costs were available for 103 CDAD cases (thereof 75 nosocomial CDAD cases). Out of 4,702 potential control patients, matching was possible for 45 nosocomial CDAD cases. Cases and controls did not differ with respect to age (p = 0.930), gender (p = 0.292), LOS on intensive care units (p = 0.463), and Charlson co-morbidity index (p = 0.902). The difference in the total LOS in the hospital showed that CDAD cases (median 27 days) stayed significant longer than their matched controls (median 20 days; p = 0.006). Overall costs for the 1,634 patient days of the 45 CDAD cases were 2,429,785 Euro (median 33,840 Euro per case) compared to 6,363,675 Euro for the 3,663 patient days for the 135 controls (median 18,981 Euro per control). CDAD cases were significant more expensive than their matched controls (median 7,147 Euro; CI95: 4,067–9,276).
Conclusion: Nosocomial CDAD is associated with high costs for healthcare systems. Clinicians should be aware of the financial impact of this disease, and application of appropriate infection control measures is recommended in order to avoid further pathogen spread.

P1471 First patient-centred, multidisciplinary clinical audit in hospital/community, study of risk factors, clinical outcomes and patient perspectives of *Clostridium difficile*-associated diarrhoea

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Objectives: Blackpool Victoria Hospital is a large DGH in NW England with two regional speciality centres. Reduction in HCAI is of top priority and has personal engagement of its chief executive. To inform designing of a comprehensive strategy for reducing rate of CDAD, the first patient centred, multidisciplinary clinical audit, enhanced to study associated risk factors, clinical outcomes (cause analysis of deaths) and patients perspective of their disease was undertaken this year across the trust/community. Contributors included patients, hospital doctors, GP and link nurses.
Method: Symptomatic *Clostridium difficile* toxin positive patients between February – April 2007 were included. When possible, Patients completed part B of the questionnaire.
Results: 63 completed questionnaires were obtained from 147 CDT+ve patients in hospital and community. 85.7% were over 65-years of age and 68.2% females. Majority of patients were from medical units including care of elderly and were admitted to hospital from their own home. 98% specimens were received in lab and 90.1% tested within 24-hrs. Oral Metronidazole used 1st line and up to 78% responded within 5–7 days. Variable non-compliance to initiation of infection prevention protocols noted. Consistent correlation of previous ceftriaxone, ciprofloxacin, co-amoxiclav, levofloxacin, imipenem, clarithromycin and PPI was observed. No association with previous clindamycin use observed. 6 surgical patients had received simultaneous ciprofloxacin /cefuroxime and metronidazole. Most patients became symptomatic within 1–2 weeks of antibiotic use. 2 patients with fulminant colitis had colectomy and 6 patients deaths unrelated/related/caused to CDAD. Patient perspectives included disease associated distress to compliments on excellent management. Variable non-compliance to infection control practices observed by patients.
Conclusion: Variable non-compliance to hospital CDAD management policy and antibiotic formulary; infection control practices; treatment of CDAD; CDT testing; duration and use of antibiotics; informing patients/relatives, and others. Action plan included engagement of directors, consultants, managers, senior nurse leads; change to 'narrow spectrum' antibiotic policy; revised CDAD management policy, and others.
A systematic feedback with a named responsible person for proposed actions and discussed at different directorates. A repeat audit is planned at the same time next year. For detailed presentation.

P1472 Factors associated with the severity of *Clostridium difficile*-associated disease: a one-year prospective study in a Lyon university hospital, France

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Objectives: The hypervirulent *Clostridium difficile* strain (NAP1/027) that emerged in North America, Japan and Europe in 2003, was related with outbreaks of severe *Clostridium difficile*-associated disease (CDAD) in North France. After a national alert in March 2006, an active surveillance was started in a French university hospital (1,000 beds) from November 1, 2006 to October 31, 2007 to describe cases of CDAD and to investigate the factors associated with a severe disease.
Methods: Hospitalised patients with either positive stool cytotoxin test or positive culture of toxin producing CD strain were included. For each case, demographic characteristics, medical history, microbiological data and risk factors for CDAD were collected according to a standardised national data collection form. Cases were classified as severe (S-CDAD) if one of the following criteria was present: #1) hospitalisation for a community-onset CDAD, #2) admission in intensive care unit

(ICU), #3) elevated leukocytosis, #4) megacolon, #5) colic perforation, #6) colectomy, #7) death within 30 days after onset related to CDAD. Severe cases were compared to mild cases using Chi2 or nonparametric tests. Multivariate logistic regression was used for risk factors identification.

Results: A total of 70 patients (47.1% male, median age of 68.5, range 1 to 96) were included. The average duration of hospital stay was 25 days (range 1 to 116). Among the 14 (20%) S-CDAD cases, 2 (14.3%) died and 2 others were admitted in ICU. The characteristics of the population are described in the table.

Abdominal pain was independently associated with a S-CDAD after multiple logistic regression (odds ratio = 12.7; [95% confidence interval: 1.4–114.4]; p = 0.02).

Conclusion: Abdominal pain is a significant factor associated with S-CDAD. The recent detection of a severe case related to a 027 strain in October 2007 in our hospital and the high proportion of hospital-acquired CDAD underline the importance of such a surveillance in healthcare settings.

Table: Characteristics of the population included in the surveillance (n = 70)

CDAD acquisition	N (%)
Hospital-acquired	31 (44.3)
Community-onset	25 (35.7)
Imported from another hospital	8 (11.4)
CDAD symptoms	
Diarrhoea	68 (97.1)
Abdominal pain	34 (48.6)
Fever >38ºC	25 (35.7)
Ileus	7(10)
Microbiological data	
Positive stool cytotoxin test	59 (84.3)
Negative cytotoxin test and positive culture	10 (14.3)
Moxifloxacin resistant strain	5 (7.1)
NAP1/027	1 (1.4)
CDAD risk factors	
Antimicrobial therapy	43 (61.4)
Laxatives	17 (24.3)
Immunosuppressive agents	9 (12.9)
Gastrointestinal surgery	3 (4.3)
Preparation before colonoscopy	1 (1.4)

P1473 *Clostridium difficile*-associated diseases: a 2-year study

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Objective: Establishing the clinical and epidemiological features of patients with *C. difficile*-associated diseases (CDAD) and the risk factors connected with such disease in our environment.

Methods: A retrospective study of patients with a positive detection of *C. difficile* toxin in human faeces between 2006–2007 in the Health Area of our hospital. Qualitative detection of *Clostridium difficile* toxins A and B was made using the VIDAS® *C. difficile* toxin A&B (Biomérieux ®) system, and clinical and epidemiological details of patients were gathered by checking their certificates of discharge.

Results: The study of *C. difficile* toxin was made on 1,289 patients and was positive in 7% (90) of total cases. Seventy-three (81%) patients were still in hospital and were 74 years old on average. 52% of them were women. Distribution according to services was as follows: 24.6% had been admitted to Internal Medicine, 23.3% to Geriatrics, 9.6% to Haematology and the rest to other services. Most of them showed underlying pathologies – 23.2% were oncological patients, 22% suffered from COPD, 19% from diabetes and 31% from other pathologies. 15% did not show any underlying disease. As to treatments prior to CDAD, 15 patients were following a cherotherapeutic treatment, 14 a corticoids treatment, 55 an antibiotic treatment and in 11 cases no treatment had been reported. The most frequent antibiotics were: β-lactams (35), quinolones (20), aminoglycosides (11) and other (9). Once CDAD was diagnosed, 64.4% of patients received a treatment with metronidazole during 10 days on average, 7% received vancomycin, 4% did not need any treatment and in 24.6% of cases no treatment was reported. 14% of patients died, but no exitus was directly related to CDAD.

Conclusion: CDAD is the most frequent cause of nosocomial diarrhoea, but our study shows that a fifth of patients come from our community – thus we can consider it an emerging community infection. β-lactams and macrolides have been traditionally reported to show a higher risk of causing CDAD but, as in other studies, we can also see that treatment with quinolones contribute to the appearance of diarrhoea. Elderly people suffering from an underlying disease are still at a high risk of suffering from CDAD.

P1474 Trends of incidence of *Clostridium difficile*-associated disease in a French university hospital between 2002 and 2006

M. Giard, P. Girardo, J. Etienne, M.E. Reverdy, P. Vanhems (Lyon, FR)

Objectives: The incidence of *Clostridium difficile*-associated disease (CDAD) first increased in North America, and was associated with the emergence of the highly virulent and antibiotic resistant strain 027. In France, the investigation of the first nosocomial outbreak due to *C. difficile* 027 highlighted the need for epidemiological baseline data. In response to a national alert, the objective of this study was to determine if CDAD incidence was increasing in a university teaching hospital.

Methods: An analysis of laboratory data collected prospectively was conducted to report the incidence of *C. difficile* toxin A detected in hospitalised patients between 2002 and 2006. The detection of toxin A was carried out on every suspected stool, while diagnosis policy did not include systematic toxin B detection. The number of admissions and number of patient-days per year were used as denominator data. A CDAD was defined as diarrhoea and detection of *C. difficile* toxin A in a clinical stool specimen. Analyses included incidence rate, number and percentage of *C. difficile* toxin A detection amongst stool specimens analysed, and population description.

Results: A total of 3,931 specimen stools were analysed for *C. difficile* toxin A detection, amongst which 284 (7.2%) were found positive. The incidence rate decreased over time (Table 1).

The percentage of *C. difficile* toxin A detected amongst all stool specimens analysed for *C. difficile* toxin A detection decreased over time ($p < 0.001$). The absolute number of *C. difficile* toxin A detected was higher in medical and surgical units but the percentage in all clinical stool specimens analysed was higher in gynaecology-obstetrical and emergency departments (Table 2).

A total of 3,363 patients had a stool specimen analysed for *C. difficile* toxin A detection, among whom 275 (8.2%) had a positive test. The percentage of women was higher amongst patients tested positive (56%) than negative (48%) (p = 0.01). Mean age of patients having *C. difficile* toxin A detected was 61.7 years. The number of *C. difficile* toxin A detection increased with age.

Conclusion: CDAD incidence does not increase over time. *C. difficile* strain 027 was detected recently in this hospital for one patient, underlying the need of epidemiological vigilance, appropriate use of antibiotics and strict implementation of infection control measures to prevent *C. difficile* spread.

Table 1. Incidence of *Clostridium difficile*-associated disease (CDAD) by year

	2002	2003	2004	2005	2006	Total
Incidence, /1000 admissions[1]	0.87	1.04	0.57	0.48	0.49	0.68
Density of incidence, /10,000 patient-days[1]	2.49	2.44	1.41	1.13	1.31	1.73

[1]Chi-square for trend: $p < 0.01$.

Table 2. Trend of *C. difficile* toxin A detection by year

Speciality	*C. difficile* toxin A detected											
	2002		2003		2004		2005		2006		Total	
	n[1]	%[2]	n	%	n	%	n	%	n	%	n	%
Medicine	41	12.2	41	8.7	26	5.2	28	5.1	28	5.6	164	7.0
Surgery	14	9.0	20	11.8	13	7.1	3	1.6	8	4.8	58	6.7
Intensive care unit	13	15.5	20	13.0	8	5.4	4	3.1	6	4.6	51	7.9
Gynaecology–obstetrics	0	0.0	2	28.6	1	12.5	1	33.3	2	20.0	6	20.7
Emergency	1	50.0	1	33.3	0	0.0	2	25.0	1	9.1	5	17.2
Total	69	11.9	84	10.4	48	5.7	38	4.3	45	5.5	284	7.2

[1]Number of *C. difficile* toxin A detected.
[2]Percentage of *C. difficile* toxin A detected above all clinical stool specimen analysed for *C. difficile* toxin detection.

P1475 Emerging disease due to *Clostridium difficile* PCR Ribotype 078 in the Netherlands

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Objective: Since the National Reference laboratory noticed an gradual increase of human *Clostridium difficile* associated disease (CDAD) due to (PCR)(Ribo)Type 078, patients data and strains were investigated in more detail.
Methods: Strains submitted to the Ref lab were characterised by PCR ribotyping and presence of toxin genes. In this study, all strains belonging to Type 078 were sequenced in tcdC region and investigated by Multiple Locus Variable Number of Tandem Repeat Analysis (MLVA). Porcine Type 078 strains (n = 30)available from another study were also included. A standardised questionnaire was used to obtain patient data.
Results: Between February 2005 and November 2007, 81 healthcare facilities submitted strains from 1553 patients to the Ref lab. Of these strains, 285 (18.4%) belonged to Type 027, 123 (7.9%) to Type 078 and 1145 (73.7%)to other types. Type 078 was the third most frequently found type. A gradual increase of type 078 strains was observed from August 2006 onwards. All 51 Type 078 strains contained tcdA, tcdB and binary toxin genes. In TcdC, a point mutation was found at position 184, resulting in a stopcodon. MLVA analysis of 20 human and 10 porcine Type 078 isolates showed 2 genetically highly related clusters. Clinical information was available from 647 of 1553 patients (41.7%). In comparison with patients with CDAD due to Type 027, patients with Type 078 were younger (67.8 years vs. 73.6 years: $p < 0.0005$)and had more often community-associated disease (27.3% vs 8.3%, OR 4.13, 95% C.I. 1.63–10.4). Severe diarrhoea was found in 40.7%, 35.6% and 27.2% of patients with Type 027, Type 078 and other types, respectively, with a significant difference between Type 027 and other types (OR 5.52, 95% C.I. 2.45–12.5). Overall mortality was 13.1%, 2.3% and 6.9% in patients with Type 027, type 078 and other types. The use of fluoroquinolones in Type 027 patients was significantly higher than in patients with other types (33.6% vs 19.5%, OR 2.60, 95% C.I. 1.24–5.46).
Conclusions: Animal and human *C. difficile* Type 078 strains are genetically highly related, supporting the hypothesis that transmission is possible. Animal and human Type 078 contained a point mutation in tcdC at position 184 resulting in a stopcodon and a defective TcdC. The clinical spectrum of CDAD caused by type 078 is intermediate between CDAD caused by Type 027 and CDAD caused by other types, but Type 078 presented more frequently as community associated disease.

P1476 Application of MLVA to study the spread of *Clostridium difficile* toxin A-negative strains of PCR ribotype 017 in a general hospital in Buenos Aires, Argentina

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Objectives: *Clostridium difficile* isolates of patients with *C. difficile*-associated disease (CDAD) usually produce both toxin A and toxin B, but an increasing number of reports mention infections due to TcdA-negative, TcdB-positive (A-/B+) strains. The finding of a cluster of A-/B+ strains in a 200-bed general Argentinean hospital resulted in a prospective study to the occurrence and spread of this variant strain using Multiple-Locus Variable-Number-Tandem-Repeat Analysis (MLVA).
Methods: From May 2000 until December 2005, faecal samples from all patients with clinically suspected CDAD were tested for CDAD. Toxin positive samples were cultured for the presence of *C. difficile*. All isolates were characterised by PCR ribotyping, toxinotyping, MLVA, presence of binary toxin genes, and alterations of toxin genes. Demographic and clinical data were collected from all CDAD patients using standardised questionnaires. Crude relative risks were estimated as odds ratios and presented with a 95% confidence interval (95% CI). All analyses were performed using the SPSS for Windows software package, version 13.0.
Results: Between 2000 and 2005, the incidence of CDAD per 1,000 admissions was 3.7 (2000), 8.4 (2001), 6.7 (2002), 4.3 (2003), 4.8 (2004) and 4.2 (2005). The annual percentage of Type 017 strains was 7.7%, 64.6%, 91.4%, 92.0%, 75.0% and 86.4%, respectively. All type 017 strains belonged to toxinotype VIII and had a 1.8 kb deletion in tcdA. Binary toxin genes were not found. Comparison of 112 Type 017 CDAD patients with 41 non-Type 017 patients revealed that Type 017 patients were more often male (68.8% vs. 46.3%, OR 2.55, 95% C.I. 1.23–5.50). Among the Type 017 isolates, 4 genetically highly related subclusters ($STRD \leqslant 2$) were identified, indicative of clonal spread. This spread showed no association in time, but was restricted to wards of Internal Medicine and Pulmonology. Of 10 patients with 21 episodes of Type 017 CDAD, 55% of recurrences concerned a different MLVA type, suggesting that the recurrent disease was not a relapse with an identical strain.
Conclusion: We conclude that *C. difficile* Type 017 gradually replaced other circulating PCR ribotypes and that MLVA was capable to recognise spread on specific wards.

P1477 Outbreak with *Clostridium difficile* PCR-ribotypes 027 and 017 simultaneously in a Dutch hospital

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Objective: At a large outbreak of *Clostridium difficile* (CD) in a 1100 bed hospital in The Netherlands, all strains were characterised and patient information collected. Predisposing factors were investigated with emphasis on previous antibiotic usage.
Methods: *C. difficile* strains were characterised by PCR-ribotyping and toxinotyping. To investigate antibiotic use in the 3 months prior to CDAD, Defined Daily Doses (DDD's), as defined by the WHO for each antibiotic were calculated per individual patient. Data on mortality were derived from the hospital database until 6 months after diagnosis of CDAD. Two groups of control patients were selected: a control group with diarhoea due to other causes than CDAD (group 1) and a control group without diarroea (group 2).
Results: Between May 2005 and January 2007, 180 CDAD patients were diagnosed; 48 (26.7%) with Type 027, 58 (32.2%) with type 017 and 74 (41.1%) with other types. All strains form Type 027 belonged to toxinotype III, whereas all Type 017 strains lacked the tcdA gene and belonged to toxinotype VIII. Control group 1 consisted of 78 patients and group 2 of 162 patients. The overall mortality was 22.9%, 21.1% and 14.3% for patients with type 027, type 017 and other types, respectively. The mortality for control group 1 and 2 was 10.5% and 6.4%, respectively. All CDAD patients used significantly more 2nd generation cephalosporins than patients of both control groups. Type 027 patients used more DDD's 3rd generation cephalosporins than patients with other types (trend: $p = 0.08$) and than patients in group 1 (trend: $p = 0.13$) and group 2 ($p = 0.03$). This same association was found for patients with Type 017. The use of fluoroquinolones was higher for Type 027 and Type 017 patients compared to patients with other types ($p = 0.08$ and 0.16, respectively) and to patients in both control groups ($p = 0.13$ and 0.005, respectively). Clindamycin was used in excess in Type 017 patients compared to patients with Type 027 and other types ($p = 0.004$

and 0.005, respectively) and patients in both control groups (p = 0.002 and 0.004, respectively).
Conclusion: In an outbreak setting, Types 027 and 017 have a high mortality, when compared to patients with other types and to control patients. The use of 2nd generation cephalosporins and fluoroquinolones was strongly associated with the occurrence of types 027 and 017, whereas clindamycin was only a risk factor for the development of CDAD caused by Type 017.

P1478 Detection of infections due to *Clostridium difficile* PCR ribotype 027 and implementation of a surveillance system for severe cases of *Clostridium difficile*-associated diarrhoea in Rhineland-Palatine, Germany, 2007

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Objectives: A recent increase in the incidence and severity of *Clostridium difficile*-associated diarrhoea (CDAD) documented in several European countries may be related, at least in part, to the emergence of a highly virulent, fluoroquinolone-resistant, PCR ribotype 027/PFGE NAP1 strain. In 2007, severe cases of CDAD caused by PCR ribotype 027 were detected in Germany for the first time. To asses the situation for this new strain two surveillance systems were established.
Methods: 1. Active surveillance for severe CDAD was established locally in a district in Rhineland-Palatine in South West Germany in which the first cases had occurred. All patients in the regional hospitals with nosocomial as well as community-acquired diarrhoea suspected for CDAD were screened for *C. difficile* PCR ribotype 027. 2. Mandatory reporting of severe cases of CDAD by clinicians was implemented nationally in November 2007. All laboratories are recommended to culture *C. difficile* from toxin positive faeces samples and to store the isolates for future characterisation and typing. Case definitions for both surveillance systems include cases with laboratory confirmed isolates of *C. difficile* ribotype 027 and probable cases (laboratory confirmation of *C. difficile* toxin A/B and defined clinical criteria for severe CDAD).
Results: Until 30th November 2007, active surveillance identified 25 probable and 14 confirmed cases in six local hospitals according to the case definition. Cases included at least four community-acquired infections. Gender distribution was 16 male and 23 female patients. Mean age was 71.8 years. Nine patients died due to a cause attributable directly or indirectly to the CDAD. Three reports of cases from two further federal states in South West Germany indicate either additional imported cases or an unexpected rapid dissemination of this strain.
Conclusion: A *C. difficile* strain with a new epidemiological profile is spreading rapidly in Germany. Further investigations are necessary in order to determine the role of potential driver's for this development such as increased fluoroquinolone use and changing age structure. Increased capacities for detection and typing of *C. difficile* as well as heightened awareness of clinicians, infection control specialists and clinical microbiologists may be required to ensure timely and adequate response to this emerging pathogen. The results confirm the importance of establishing an effective surveillance system in a timely manner on a national level.

P1479 Surveillance of *Clostridium difficile* NAP1/O27 since 2006 in Belgium

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A European prospective study (Barbut et al. 2005) revealed the presence of *Clostridium difficile* NAP1/O27 strains in the Netherlands, Ireland and Belgium. Since
January 1st 2006 an official reference centre was created in Belgium. The aim of the centre is to help hospitals in managing *C. difficile* outbreaks and in identifiying and typing their strains.
Objective: We organised a surveillance program of *C. difficile* associated disease (CDAD) in Belgium, which started in January 2006. The aims were to detect *C. difficile* clusters, to establish the prevalence of the NAP1/O27 clone, and to prospectively assess the incidence of CDAD in acute care hospitals according to a standardised protocol established by the Belgian Infection Control Society.
Methods: Each participating laboratory was asked to send strains in case of clusters, and for the prospective component, to report monthly denominator data as well as clinical data on every case to the Scientific Institute of Public Health and to send the first five *C. difficile* isolates every six months to the reference laboratory.
Strains were analysed, after species confirmation, for tcdC deletion (RT-PCR), binary toxin (RT-PCR), toxinotyping (PCR B1-A3 + RFLP) and ribotyping (RT-PCR). On 92 strains typed as NAP1/O27 we measured minimal inhibitory concentrations of Ciprofloxacin and Moxifloxacin by use of E-test.
Results: From January 2006 until end of october 2007 we confirmed 1401 strains from 93 centres, as *C. difficile*; 338 strains (24.1%) from 47 centres (50.5%) belonged to ribotype NAP1/O27. The second most prevalent ribotype was ribotype 3 (Brazier's 078) with 68 isolates (5.1%). All 92 strains tested for Moxifloxacin and Ciprofloxacin susceptibility and all of them were resistant to both drugs. The incidence of nosocomial CDAD (onset >2 days of hospital stay) in 32 hospitals having participated to 2 semesters of the prospective surveillance was 1.6 cases per 10000 patient days (95% CI 1.0–2.1) in the second semester of 2006 and 0.9/10000 (95% CI 0.6–1.2) in the first halfyear of 2007.
Conclusions: The NAP1/027 clone has spread all over the country, accounting for 24% of the strains. It is present in more than 50% of the hospitals. In some centres it was clearly implicated in outbreaks but a lot of sporadic cases were also observed.

P1480 Risk factors for *Clostridium difficile* ribotype 027 infection in Germany: preliminary results of a retrospective case-control study

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In 2007, severe cases of *Clostridium difficile* associated diarrhoea (CDAD) caused by *Clostridium difficile* ribotype 027 were detected in Germany for the first time. Initially, patients on several wards in a hospital in southern Germany were affected. Comprehensive investigations were launched by the local and national health authorities in order to determine and control the spread of infections due to this emerging pathogen. To assess the possible risk factors for severe CDAD, an analytical study was performed.
A retrospective case-control study was conducted in the index hospital. Cases with laboratory confirmation of *C. difficile* ribotype 027 and probable cases (laboratory confirmation of *C. difficile* toxin A/B and defined criteria for severe CDAD) were included. Controls were matched (aiming for a rate of 1:3) for ward, date of admission, and length of stay. Univariate and nonparametric data analysis was performed using STATA 9.
Two confirmed cases, 4 probable cases and 16 controls were included. Severe CDAD was strongly associated with receipt of antibiotics in the three month before onset of symptoms (MOR n.d.; $p < 0.01$), namely third generation cephalosporins (MOR n.d.; $p < 0.01$) and fluoroquinolones (MOR n.d.; $p < 0.01$). Admission to the dialysis ward in the 3 months before the index admission was also associated with infection (MOR 8.0; $p = 0.03$). Undergoing endoscopy, surgery, use of proton pump inhibitors and hospitalisation in the last three months were not significantly associated with the disease.
Although these data are based on a small sample size (recruitment is ongoing), the preliminary results suggest that severe CDAD is strongly related to the use of high-risk antibiotics, i.e. fluoroquinolones and third generation cephalosporins. Control of CDAD may be attained by minimising the administration of these antibiotics. In addition, our results indicate transmission of *Clostridium difficile* especially among dialysis patients. The frequency of patient visits and prolonged physical contact with the environment during dialysis treatments make these facilities potentially efficient settings for nosocomial pathogen transmission.

Isolation measures and adapted infection control practices are warranted to reduce nosocomial transmission of CDAD in dialysis units.

P1481 An outbreak of *Clostridium difficile*-associated disease in a university hospital in Germany

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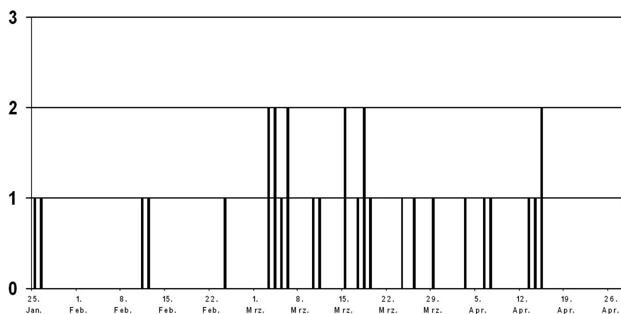
Objective: The incidence of *Clostridium difficile*-associated disease (CDAD) in Germany is increasing, but outbreaks have been reported very seldom in the past. We investigated an outbreak in a surgical department of our university hospital during January through April 2007.

Methods: A case-control study and a multivariate analysis were performed. A case was defined as the onset of diarrhoea $\leqslant$48 h after admission and (b) either detection of *Clostridium difficile* (CD) toxins A or B in stool specimens by ELISA or culturing of toxin-producing CD. Severe CDAD was defined as non-selflimiting course of disease. All other patients admitted to the same unit during this time period developing diarrhoea but failed detection of CD served as controls. Age, gender, underlying diseases, and nosocomial acquisition of diarrhoea prior antimicrobial treatment were assessed. Culture-positive samples were investigated by pulsed-field electrophoresis (PFGE) using Gelcompar II (R) software. PCR-ribotyping was performed to test for the hypervirulent strain O27. Antimicrobial resistance towards fluoroquinolones, cephalosporins, penicillins, clindamycin, linezolid, aminoglycosides and glycopeptides was determined.



Cases of CDAD (n = 31).

Results: 31 patients with CDAD were involved. No severe cases of CDAD were found. 28 patients were included in the control group. According to multivariate analysis age (OR 7.05, 95% CI: 1.57–50.37) and the treatment with fluoroquinolones (FQ) (OR 9.23, 95% CI: 1.42–182.40) showed to be significant risk factors for development of CDAD. By PFGE 11 of the 12 outbreak isolates were undistinguishable. The predominant strain was ribotype 001, which is the predominant strain in Germany. The outbreak strain showed resistance towards ciprofloxacine, levofloxacine and moxifloxacine.

Conclusion: Exposure to FQ was a significant risk factor for CDAD acquisition in our study. Restrictive use of antimicrobial substances including FQ, prospective surveillance of CDAD and appropriate infection control measures are recommended to prevent the transmission and the onset of CDAD.

P1482 Comparison of toxigenic status and PCR ribotype of *Clostridium difficile* in three university teaching hospitals in Korea

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Objectives: *Clostridium difficile* is the main aetiological agent of antibiotic-associated diarrhoea and the most common cause of infectious hospital-acquired diarrhoea. Toxins A (enterotoxin; TcdA) and B (cytotoxin; TcdB) are well known primary virulence factors of *Clostridium difficile*. Toxigenic strains of *C. difficile* usually produce both toxin A and B, although toxin A-negative, toxin B-positive strains have been responsible for *C. difficile* associated disease (CDAD). Some strains of *C. difficile* also produce a binary toxin. The prevalence of strains of *C. difficile* producing toxin A, toxin B, and binary toxin has been reported to vary geographically but has rarely been studied in Korea. The aims of this work were to compare the toxigenic status of *C. difficile* isolates in three teaching hospitals and to evaluate the characteristics of molecular type by PCR ribotyping.

Methods: A total of 162 unduplicated isolates of *C. difficile* recovered from patients with diarrhoea in three teaching hospitals in 2007 were studied. The three hospitals are triangularly located in Seoul and the distance is 10 to 20 km each other. PCR was used to amplify genes for toxin A (tcdA), toxin B (tcdB), the repeating sequence of toxin A (tcdA rep) and binary toxin (cdtA, cdtB). PCR ribotyping was performed in all isolates.

Results: The isolation rates of *C. difficile* in A, S, and Y hospitals were 4.6% (20 isolates/437 cultures), 8.4% (140/1533), and 3.7% (13/347), respectively. Of the 162 isolates tested, 131 (80.9%) were toxigenic strains. The proportions of toxin A-negative, toxin B-positive strains in A, S, and Y hospitals were 20%, 35.6%, and 15.4%, respectively. The proportions of binary toxin gene positive strains in A, S, and Y hospitals were 20%, 5.4%, and 15.4% respectively. All binary toxin-producing strains were toxin A and B positive. Thirty-five ribotypes were identified by PCR ribotyping. A+B+ isolates comprised 25 ribotypes. All A-B+ strains showed the same banding pattern. There was no predominant ribotype in A and Y hospitals. In S hospital, the most common type (except toxin A-B+ isolates) accounted for 28% of isolates.

Conclusion: The prevalence of toxin A-B+ and binary toxin gene-positive strains differ from the three hospitals in one city. The predominant strains such as toxin A-B+ may contribute to high prevalence of *C. difficile*. Further nationwide multicentre surveys are needed to ensure the prevalence of those strains in Korea.

P1483 First cluster of *Clostridium difficile* PCR ribotype 027 in Denmark

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Objectives: *Clostridium difficile* PCR ribotype 027 (CD027) has been associated with outbreaks and severe clinical manifestations in North America and Europe. National surveillance of *Clostridium difficile*-associated disease (CDAD) in Denmark was initiated in 2007 after the first detected cluster of CD027 in the western part of Denmark.

Methods: Isolates of *C. difficile* received from hospitals were analysed by PCR to detect the toxin genes tcdA, tcdB and cdtAB. Isolates positive for these toxin genes were investigated by the following analyses: (i) PCR and sequencing in order to detect internal deletions in the tcdA gene and the toxin-regulating gene, tcdC, (ii) PCR ribotyping, and (iii) Susceptibility to relevant antibiotics by Etest.

Results: In the period of November 2006 to Marts 2007 a cluster of ten *C. difficile* cases were analysed originating from three hospitals within a region in the western part of Denmark. Seven of these cases were identified as CD027. Subsequent surveillance from June to August 2007 revealed five additional CD027 cases out of 24 submitted isolates of *C. difficile* from that particular region.

All CD027 cases were tcdA, tcdB and cdtAB positive, had no internal deletions in tcdA and a tcdC gene with an 18bp deletion and a one-base pair deletion at position 117.

Antimicrobial susceptibility testing showed that all CD027 cases were sensitive to erythromycin, vancomycin, metronidazol and clindamycin and resistant to ciprofloxacin and moxifloxacin.

One non-CD027 isolate from the cluster was identified with a tcdC deletion at position 117. This strain was tcdA, tcdB and cdtAB positive, had no internal deletions in tcdA, a tcdC gene with an 18bp deletion and the same antimicrobial resistance profile as CD027 except it was susceptible to moxifloxacin.

No immediate epidemiological link between the patients with CD027 infection was observed.

Conclusion: This is the first report describing CD027 in Denmark. These CD027 strains harboured toxin genes, toxin gene deletions and displayed antimicrobial resistance profiles characteristic of the hypervirulent CD027 previously seen in North America and other parts of Europe.
It is important to improve the surveillance of CD027 and other emerging PCR ribotypes in order to understand the epidemiology of *C. difficile* infections and enhance the infection control.

P1484 Molecular epidemiological analysis of *Clostridium difficile* infections in a Swedish County

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Objectives: This study was conducted to evaluate the occurrence of *Clostridium difficile* associated diarrhoea (CDAD) in a Swedish county. Furthermore, we investigate where the episodes occurred, and however possible nosocomial transmissions of certain *Clostridium difficile* ribotypes could be detected.
Methods: During one year from September 2000 through August 2001, all *Clostridium difficile* toxin positive faecal samples in the county of Jönköping were cultured and isolates were ribotyped using a PCR based method. Clinical and epidemiological data was then collected by evaluating medical records of each patient.
Results: We detected a total of 360 episodes in 284 patients (109 episodes/100.000 inhabitants) of CDAD, and a large proportion (36%) of primary episodes was community acquired. A total of 32 different ribotypes were identified, and in concordance to previous Swedish studies, the most common ribotypes were SE16, SE17, SE20 and SE21. We did not detect the virulent ribotype O27. Ribotypes SE17 and SE20 were often associated with hospital acquired CDAD, and these types had the highest transmission rates. The main part of possible transmissions occurred when patients were hospitalised on the same ward during the same time period, indicating that the hospital environment is less important as a bacterial reservoir for transmission.
Conclusion: Some ribotypes of *Clostridium difficile* seems to be more virulent than others. In our study, we found SE17 and SE20 to be more virulent, and others have shown this for ribotype 027. By using ribotyping, transmission data can easily be evaluated. In addition, ribotyping is of value to monitor the occurrence and spread of more virulent isolates. Thus epidemiological and hospital infection control measures can be improved to minimise spread.

P1485 Surveillance of *Clostridium difficile* infection in Latvian multidisciplinary teaching hospital

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Introduction: *Clostridium difficile* (CD) is the common causative agent of nosocomial diarrhoea. A new highly virulent strain (ribotype 027) has emerged in many European countries. The study was conducted to determine the incidence and main risk factors of CD associated diarrhoea in the large multidisciplinary teaching hospital in Latvia.
Methods: The retrospective analysis of clinical records of all CD cases starting in 2004–2006 was performed. A CD associaciated diarrhoea (CDAD) case was defined as diarrhoea in a hospitalised patient with a stool specimen that tested positive with Culturette CDT latex agglutination (2004–2006) or Toxin A test (2007) tests. A questionnaire was completed for all CDAD patients. In 2007 prospective surveillance of CDAD was initiated. Starting from Sept 2006 CD was cultured and subjected to ribotyping, CD DNA was extracted from stool and the presence of the tcdA and tcdB genes was established by PCR.
Results: A total of 112 patients with CDAD were identified. Their age varied from 20 to 94 years (mean 58.8) and 60.7% were female. The overall 3 years (2004–2006) incidence was 9.6 cases per 10 000 admissions with highest annual incidence in 2004 – 16, 1 cases par 10 000 admitted patients. No death, toxic megacolon or perforation due to CDAD was reported during the study period. Nine patients (8%) had pseudo-membranous colitis confirmed by colonoscopy, 62, 5% of patients had fever. The most frequent risk factors identified were use of antibiotics (89%), previous hospitalisation in last 6 months (47%) and antacid use (57%).
Thirty-two percent of the patients with CDAD were identified in nephrology ward, 15.2% in gastroenterology ward and 7% in transplantation unit.
From Sept 2006 to Sept 2007 20 CD (tcdA+, tcdB+) isolates were ribotyped. 14 isolates displayed different patterns while 6 isolates were identical according to their ribotype. Identical isolates originated from nephrology and thoracic surgery wards. None of the isolates belonged to ribotype 027.
Conclusions: CD infection in our hospital was associated with relatively low morbidity and mortality. The highest incidence was reported in the department of nephrology. Clonal analysis indicated the presence of local outbreaks involving several departments. The new emerging type 027 was not identified during the active surveillance. Active surveillance and infection control guidelines for outbreak containment should be implemented in order to avoid the rapid spread of the new strain.

P1486 Clinical-epidemiological characterisation of *Clostridium difficile* – ribotype 053

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Objectives: *Clostridium difficile* associated disease (CDAD) has been changing in its clinical severity and epidemicity. Hypervirulent strains of *Clostridium difficile* (C.diff.) are causing hospital outbreaks in Europe. This phenomenon may not only be due to PCR ribotype 027. Hospital surveillance programs should therefore not focuse only on this ribotype but also on other potential hypervirulent strains of C.diff. The aims of our surveillance program are to monitor incidence and death rate of CDAD in our hospital, to characterise selected clinical cases by ribotyping and to asses the efficacy of infection control precautions.
Methods: Since February 2006 an active clinically and laboratory based surveillance program has been in place. All patients with diarrhoea are obligatory screened for CDAD. Case definitions according to the *Clostridium difficile* working group recommendations (ECDC Advisory Forum 2006)were used for case identification and classification. Data collection was done until October 2007.
Results: 514 patients fulfilled the definition criteria of a case of CDAD in a period of 19 months. Of these 514 cases 91 were selected because of a severe course of their CDAD episode, relapse of CDAD and because of having been related in time and/ or space with the severe or relapse cases. All 91 cases were retrospectively subjected to extensive epidemiological and molecular biological investigation including PCR-ribotyping. There was no ribotype 027 found among the isolates from the 91 cases. The most prevalent ribotype found was ribotype 053 (45; 49.5%), whereas 20 of these cases showed a fatal course (44.4%). Ribotype 053 showed a high case-fatality-ratio (0.4) and was also suspected to be highly transmissible. It was responsible for at least two clusters in 2 neighbouring wards in April and May 2007. We were also able to demonstrate twice a survival of Ribotype-053-spores in the hospital environment close to 053 positive patients despite efficacious routine desinfections measures. For that reason ribotyp 053 should be considered as a strain of *Clostridium difficile* with high transmissibility and high virulence.
Conclusions: Based on ribotyping one major strain was identified in a surveillance period of 19 months. Our findings indicate a high transmissibility and hypervirulence of strain Ribotype 053. Further efforts should be put on molecular-microbiological investigations of this strain and its toxins detecting responsible virulence factors.

P1487 A national service to determine ribotypes causing *Clostridium difficile* infection: *C. difficile* Ribotyping Network for England (CDRNE)

M. Wilcox, W. Fawley for the CDRNE Working Group

Objectives: A Health Protection Agency funded network of 6 laboratories (CDRNE) was established in April 2007 to provide access for hospitals throughout England to *C. difficile* culture and ribotyping, according to standardised criteria for submission of faecal samples. We report results from the first phase of CDRNE activity.
Methods: The CDRNE consists of 6 regional microbiology laboratories in England: Leeds (Reference Laboratory), Birmingham, London, Manchester, Newcastle and Southampton. The service is centrally funded, and so referring laboratories/hospitals are not charged. The CDRNE service is for use by hospitals/infection control teams in England to investigate: increased frequency of cases or high baseline rates of CDI; increased severity/complications of CDI cases; increased mortality associated with CDI; or increased recurrence rate of CDI. We aim for a 1–2 week turnaround time to culture and then ribotype CD strains from submitted faecal samples, according to a standardised UK method. A minimum data set is submitted with each sample to provide reason for referral, antibiotic risk and outcome data.
Results: 670 CD toxin positive samples from patients with CDI were processed in the first 7 months of the CDRNE service roll out. CD culture yield for the mailed faecal samples was ~90%. CDRNE samples represented approximately 1 in 40 of the expected number of CD toxin positive cases in England, based on data for the previous 6 months. The overall proportions of the 3 most common CD ribotypes detected, 027, 106 and 001 were 45%, 15% and 11%, respectively. There was a marked regional variation in the prevalence of CD ribotypes: for example, 027 (8–63%), 106 (3–30%) and (0–30%) 001. The next most common ribotypes were 002 and 023 (up to 12% and 17% regional prevalence, respectively). Comparison of CDRNE data with those collected for all CDI cases in Leeds shows the potential for sampling bias, likely reflecting the submission criteria.
Conclusion: CDRNE has provided increased access to ribotyping as part of the investigation of increased incidence, severity or complications associated with CDI. Marked variation in the prevalence of ribotypes needs to be explained with particular reference to the transmission of clones and sub-clones within institutions, possibly using additional highly discriminatory DNA fingerprinting methods.

P1488 Community-onset *Clostridium difficile*-associated diarrhoea: is it truly community-acquired?

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Objectives: The incidence and clinical severity of nosocomial *Clostridium difficile*-associated disease (CDAD) is on the rise, partly because of outbreaks due to the hypervirulent strain ribotype 027. Little is known of the incidence of community-onset CDAD (CO-CDAD), nor of its characteristics as compared with nosocomial CDAD. Herein, we investigate CO-CDAD in 3 areas in the Netherlands.
Methods: Three regional Microbiology Labs tested all samples of unformed stool sent in through general practitioners (GPs) during 3 months for presence of *C. difficile* toxins by enzyme immunoassay. In case of a positive toxin test, patients were enrolled in the study and completed a questionnaire on clinical characteristics of the diarrhoea, contact with hospitals or nursing homes, prior antibiotic usage or other medication, co-morbidity and possible animal contacts. Toxin-positive stools were cultured for *C. difficile* and characterised by PCR ribotyping.
Results: Over 2200 loose stool samples were analysed of which 41 (1.9%) were toxin positive and *C. difficile* confirmed by culture. Preliminary analysis shows that the mean age of CO-CDAD patients was 59 (range 1 to 91) yrs; 11% were under 20. Diarrhoea usually was long-lasting and severe, with a mean duration of 17 days, a watery consistency in 93%, admixture of blood in 50%, a frequency of more than 10 times daily in over 50%, fever in 43% and abdominal pain in 86% of cases. Part was in line with the GPs standard to order stool tests only when diarrhoea is severe or lasts 7 days or longer. About half of the cases lacked any association with healthcare institutions. Half reported usage of antibiotics in the past year, whereas 50% regularly consulted a medical specialist for co-morbidity. About 64% of patients used some medication chronically.
Strains differed clearly from hospital strains and belonged to various uncommon PCR ribotypes. Frequently found nosocomial PCR ribotypes 078 and 014 were seen in CO-CDAD in 13% of typable strains each.
Conclusion: *C. difficile* toxin can be demonstrated in up to 2% of stool samples from severe and/or lasting community-onset diarrhoeal disease. In these subjects, age, co-morbidity and prior use of antibiotics varies greatly. Less than half of the cases can be related to healthcare institutions or antibiotic usage. In accordance herewith, ribotypes of community-onset *C. difficile* strains differ from common nosocomial ribotypes, both suggesting that there is a reservoir in the community.

P1489 *Clostridium difficile* at a teaching hospital in Western Australia: providing insight into community acquisition

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Objectives: To describe cases of *C. difficile*-associated diarrhoea (CDAD) diagnosed at Royal Perth Hospital (RPH) by demographics, place of acquisition and risk factors.
Methods: Setting: A 700-bed adult teaching hospital in Western Australia.
Design: A retrospective chart review of laboratory-confirmed CDAD diagnosed at RPH between July 2003 and August 2007. CDAD was defined on the basis of a positive culture and toxin detection (EIA A or B). Two positive tests for *C. difficile* greater than 3 months apart in a given patient were considered separate episodes.
Main outcome measures: demographics, place of acquisition, antibiotic use for previous 3 months, comorbidities, gastrointestinal medications and procedures.
Results: A total of 193 episodes of CDAD were identified in 189 patients, with 53% female and a mean age at diagnosis of 63 years (range 13–94 years). Medical records were reviewed for 184 patients. Most cases were associated with hospitalisation: 108 (59%) during the admission at RPH, 36 (19.6%) during a previous admission at RPH and 10 (5%) during admission at another hospital in Western Australia. However, there were 30 cases (16%) with no record of prior hospital admission in the 3 months before onset of symptoms. RPH cases had a higher mean age-adjusted Charlson Comorbidity Index (4) compared with community cases (2.5). Overall, 22% of cases were not associated with antibiotic use. Community cases were less likely to have been exposed to antibiotics than RPH cases (14% vs.67%, $p < 0.001$). Piperacillin-tazobactam (24%), ticarcillin-clavulanate (21%), amoxicillin-clavulanate (19%), ciprofloxacin (18%) and cephazolin (17%) were the most frequently implicated antibiotics. The main antibiotic classes used were penicillins 71(39%), first generation cephalosporins, 39 (21%) and fluoroquinolones 37 (20.1%).
Conclusions: Community acquisition of *C. difficile* may be more prevalent than previously thought. Just under one fifth of all cases of CDAD diagnosed in this tertiary hospital were community acquired and this is likely an underestimate of prevalence. Diagnostic laboratories serving community patients need to be aware that *C. difficile* may be a significant cause of diarrhoea in this setting. Although not traditionally considered high risk for *C. difficile*, piperacillin-tazobactam and ticarcillin-clavulanate were the most common antibiotics implicated, suggesting healthcare facilities need to evalute their own data when looking at risk factors.

P1490 Is the emergency department the place to be for optimum *Clostridium difficile*-associated disease control?

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Background: Alerted by the rise and severity of nosocomial *Clostridium difficile* (CD)-associated disease (CDAD) and the high suspicion of import diarrhoea, a tertiary care teaching hospital set up a hospital-wide infection control (IC) programme, aiming at increasing CDAD-detection, improving standard precautions, organising IC practitioner rounds, environmental disinfection with hypochlorite, early contact isolation and peroral vancomycine antibiotic treatment. To date CD ribotype 027 in Belgium accounts for 23% of all strains tested.
Objective: To identify the importance of the emergency department (ED) as an entry point of CDAD, since its case mix consists of patients at high risk of both introducing and acquiring infections.
Methods: A retrospective study of CDAD was conducted reviewing ED admission and discharge data of 4960 patients presenting with diarrhoea between January 1, 2002 and November 15, 2007. Variables included are epidemiological (age, sex, origin, dismissal) as well as bacteriological (CD-culture and -toxin analysis).
Results: A total of 1926 CD-analyses were performed, showing a mean total detection effort of 40%, yet increasing over the study period: 34% (2002), 32% (2003), 35% (2004), 40% (2005), 41% (2006) and 53% (2007). Of these, 166 (originating from 146 patients) were found CD-toxin positive, indicating a CD-recovery rate of 9%, which is even threefold the overall hospital detection yield.
Of the studied ED patients 27% were aged above 65, representing 68% (99/146) of the CDAD affected. Hospital admission for diarrhoea (3068 out of 4960) accounted for 62%. Only 4.5% were transferred from long-term care facilities. There was no sex difference observed.
Conclusion: Increasing ED awareness and a high CD-recovery rate stress the importance of the ED in expediting case detection.
The high hospitalisation rate of patients presenting with diarrhoea and a clear import pattern moreover urge for early prevention of transmission by timely syndromic application of contact precautions. Above 65 years of age, quarantine may be warranted in all diarrhoeic patients, yet not workable. Isolation based on risk factors (comorbidity, previous antibiotic or antacid exposure or CDAD history) seems more feasible. Therefore, this case mix of ED patients needs more refinement.
In the mean time, clinical condition based IC practices should emphasise on hand hygiene, high touch surface disinfection and careful selection and handling of medical items.

P1491 Outbreak of *Clostridium difficile*-associated disease in Luxembourg

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Since 2003 outbreaks of *Clostridium difficile* associated disease (CDAD) have been recognised in Canada and United States and thereafter in Europe. These outbreaks are caused by a new ribotype called PCR 027. During the summer 2006 we noticed an increase of the number of cases of CDAD in our hospital. At that time stool culture was not performed, stools were tested for antigen and toxins A and B. After the first of September 2006 stools were cultured to isolate CD and ribotyping was done by a central laboratory. Between the 1st of September 2006 and the 31st of August 2007 106 cases of CDAD were identified. The mean age was 63, there were 53 men and 53 women. Eighty-one strains were isolated from diarrheic patients with suspected CDAD, 27 strains were ribotype 027. Sixteen patients died from CDAD or underlying disease, seven infected with CD 027.
CD ribotype 027 has reached Luxembourg probably in winter or spring 2006 and has spread rapidly causing a large outbreak in our hospital. Mesures have been taken to avoid the spread from patient to patient with reinforcing the isolating procedures.

P1492 Microbiological surveillance of *Clostridium difficile* infections in Luxembourg, 2006–2007

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Objectives: *Clostridium difficile* infections in the elderly have emerged as major public health problem in many countries including Luxembourg. The National Health Laboratory department responsible for the surveillance of enteric pathogens started a prospective microbiological surveillance of *Clostridium difficile* strains in October 2006 in collaboration with hospital and private laboratories. Here we present first results from this national surveillance scheme including ribotyping, demographic characteristics, and mortality of patients with laboratory confirmed *Clostridium difficile* infection.
Methods: Since October 2006, all received *Clostridium difficile* strains were ribotyped by PCR. Since July 2007, PCR detection of clostridial toxin genes tcdA, tcdB and binary toxin genes cdtA and cdtB was added for further strain characterisation. Routine demographic data (age, sex) and vital status of laboratory confirmed cases was obtained from the National Registry System. Mortality was calculated based on date of strain referral and date of death. A logistic regression model was used to assess risk factors independently for mortality.
Results: In the first 12 months of the microbiological surveillance, we received strains from 223 patients with laboratory confirmed *Clostridium difficile* infection, originating mainly from hospital laboratories, compared to 151 strains of *Salmonella* and 435 strains of *Campylobacter* received during the same time period. 68 strains (30.5%) had ribotype 027. Median age of patients was 63 years but differed significantly ($p = 0.001$) between patients with ribotype 027 (74y.) and patients with other ribotypes (59y.). No relation between sex and ribotype was observed ($p > 0.05$). The mortality rate within the month and within 3 months of strain referral was 14.8% and 21%, respectively. In a logistic regression model, one month mortality was significantly associated with advanced age ($p < 0.0001$), but not with sex ($p = 0.66$) or ribotype ($p = 0.14$).
Conclusion: In Luxembourg, *Clostridium difficile* has emerged as a major enteric pathogen responsible for substantial morbidity and mortality among the elderly. Ribotyping is a useful technique for subtyping strains, but is handicapped by the lack of availability of a curated online database of ribotype profiles. Microbiological surveillance is essential to detect clusters of cases or to monitor the spread of different clones through time.

P1493 Molecular analysis of *Clostridium difficile* strains isolated from healthy horses in the area of Parma, Italy

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Objectives: *Clostridium difficile* has been associated with a wide spectrum of diseases in humans and several animal species. In particular, *C. difficile* is an important cause of colitis in adult horses and foals. In horses, faecal carriage rates of 2–29% have been reported. The aim of this study was to investigate the molecular characteristics of *C. difficile* strains isolated from healthy horses by using toxin gene profile.
Methods: A total of 42 healthy horses (24 adults and 18 foals <14 days old) faecal samples was tested for the presence of *C. difficile* toxin A and B with a commercial enzyme immunoassay (Remel). *C. difficile* was isolated onto cycloserine-cefoxitin-fructose agar (CCFA) before and/or after thermal shock. Preliminary identification of *C. difficile* was based on colonial appearance, odour, lack of aerotolerance and cell morphology after Gram staining. Final identification was performed by using a rapid latex agglutination test (OXOID) and Rapid ID32A (bioMérieux) biochemical profiles. *C. difficile* DNA was extracted by boiling colonies in distilled water and used as a template for screening of genes encoding for toxin A, B and binary toxin (CDT). PCR detection for tcdA and tcdB genes was performed as previously described by Spigaglia and Mastrantonio (2002), and the presence of CDT was tested by amplification of cdtA and cdtB genes as described by Stubbs (2000). Toxigenic strains were tested for in vitro production of toxins by EIA.

Results: *C. difficile* strains were isolated from 14 of 42 faecal samples (33%): 6 from 24 adult horses (25%) and 8 from 18 foals (44%). Six (43%) of 14 isolates possessed genes encoding at least one toxin: one (a foal) possessed tcdA and tcdB genes and 5 possessed only tcdB gene (3 adult horses and 2 foals). Eight isolates lacked tcdA and tcdB genes. None of *C. difficile* strains tested possessed ctdA and ctdB genes. Only 1 of the 42 (2.4%) faecal samples tested for toxins A/B by EIA was positive. The *C. difficile* strain isolated from this sample resulted tcdA-/tcdB-. All PCR-positive strains resulted negative for in vitro toxin production by EIA.
Conclusion: The results of this study suggest that *C. difficile* could be present in the normal intestinal flora of adult horses, not only in foals. By PCR it was demonstrated that healthy horses can serve as a potential reservoir of toxigenic *C. difficile*.

P1494 Screening for *Clostridium difficile* in meat from French retailers

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Clostridium difficile is well known as a human pathogen, but it is also responsible for colonisation or infection in a wide range of animal species. It has been shown that *C. difficile*-associated disease is an important problem in horses, pigs, piglets and calves. Consequently, concern has been raised about contamination of food products. Recently, *C. difficile* has been isolated from retail meat in North America. The relevance of this presence is unclear but food animal may be a source of contamination for humans.
Objectives: The aim of this work was to assess the presence of *C. difficile* in retail meats in France.
Methods: Meat samples were collected from different retailers and included 65 ground beef samples (vacuum-packed or not) and 50 pork sausage samples. Five grams of each sample were suspended in 100 ml brain heart infusion containing 0.1% taurocholate, cycloserine and cefoxitine (CCT BHI). After incubation in anaerobiosis at 37°C for 72 h, two aliquots were subcultured on Taurocholate Cycloserine Cefoxitin Agar (TCCA), one of which after alcohol shock.
Results: A total of 115 meat samples were studied. *C. difficile* was isolated from 3 of 65 (4.6%) ground beef samples. None was detected from pork sausages. The 3 isolates were further characterised by antibiotyping, toxinotyping, detection of binary toxin and PCR-ribotyping and compared to human strains isolated in France during the same period. Among the three isolates, two belonged to toxinotype 0 (binary toxin negative, moxifloxacin susceptible) and one to toxinotype III (binary toxin positive, tcdC deletion −18bp, moxifloxacin resistant).
Conclusion: Further studies are underway to confirm these preliminary data and to assess the prevalence of animal food contamination by *C. difficile*.

P1495 *Clostridium difficile* PCR ribotype 078 toxinotype V in Dutch food-producing swine

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Objectives: From recent *Clostridium difficile* (CD) outbreaks in human populations with high morbidity and mortality the question was raised whether animals may play a role as a vector. PCR ribotypes of CD identified in humans have indeed been found recently in pigs and calves as well. Here, the occurrence of CD in Dutch pigs destined for pork production, was investigated in diarrhoeal animals.
Methods: Two pig breeding farms with neonatal diarrhoea for more than a year were selected for sampling. A one-strip assay (ICTAB) was used to screen animal faeces for toxins A and B. Toxin-positive faeces were cultured for the presence of *C. difficile* and isolates were characterised by PCR ribotyping and multi-locus-variable-tandem-repeat analysis (MLVA). A control group of 17 type 078 isolates obtained from patients with nosocomial CD-associated disease (CDAD) in the period 2006–2007, was included.
Results: *C. difficile* was not detected in seven unaffected farms, but was detected in 1 to 4 days-old diarrhoeal piglets with colitis (6 out of 12 litters in total) in two farms with persistent neonatal diarrhoeal outbreaks. Surprisingly, the mother sows (N = 12) were apparently healthy and ICTAB-negative. Isolates were identified as PCR ribotype 078 harbouring toxin A (tcdA) and B (tcdB), tcdC and binary toxin genes, but free from the ermB gene. The tcdC gene showed a 39 bp-deletion. Application of MLVA on ten pig strains revealed that all strains were genetically related (STRD < 10) with only variation in 1 of 7 loci tested. Of 17 human isolates, 15 also belonged to this same cluster.
Conclusion: This is the first finding of *C. difficile* PCR ribotype 078, toxinotype V in an animal matrix in The Netherlands. The strains were genetically related to human isolates of the same PCR ribotype. It is, therefore, now crucial to assess the risk of this potential zoonosis in the community.

P1496 *Clostridium* species found in Norovirus outbreaks in the Netherlands

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Objectives: Outbreaks (OBs) of acute gastroenteritis (AGE) of suspected viral aetiology are reported to RIVM as part of the Norovirus (NoV) OB surveillance system. Since 2002, the epidemiology of NoV has changed. Simultaneously, the emergence of a variant of *Clostridium difficile* (CD) ribotype 027, which appeared to have increased pathogenicity, was reported. The coincidental increase in NoV OBs and emergence of CD ribotype 027 raised the question if these events could be related. In theory, an episode of NoV illness can result in increased growth of *Clostridium* spp. and subsequently lead to more severe or prolonged illness. Additionally, spread of *Clostridium* spp. could be increased by multiple episodes of vomiting and diarrhoea caused by NoV infection. Therefore, we studied the prevalence of CD in OBs of AGE caused by NoV, rotaviruses (RV) and of unknown aetiology.
Methods: Stool samples of OBs caused by NoV, RV, and of unexplained aetiology were tested by Premier *C. difficile* Toxin A&B EIA, positive samples were tested for the presence of CD by culture methods using an ethanol shock pretreatment. Suspected colonies were tested using CD-specific GluD and ribotyping PCR. Negative samples were further tested using 16S rRNA PCR.
Results: See the table.

Table 1. Number of *Clostridium* spp. positive samples and OBs of unexplained, NoV and RV AGE OBs.

Study population	Group 1	Group 2	Group 3
Description	unexplained GE	NoV	RV
N (specimens/OBs)	120/34	350/56	98/21
Positive EIA (specimens/OBs)	1/1	16/11	l/1
Positive culture (specimens/OBs)	l/1	15/10	1/1
Positive morphological criteria (specimens/OBs)	1/1	13/9	1/1
Positive GluD PCR (specimens/OBs)	1/1; *C. difficile* 001	0/0	0/0
Positive 16S PCR (specimens/OBs)	1/1	13/9	1/1
Results 16S PCR	*C. difficile*	*C. disporicum* *C. perfringens*/*C. disporicum* *C. disporicum* *C. disporicum* *C. subterminale* *C. boltei* *C. butyricum* *C. bifermentans* *C. disporicum* *C. sordellii* *C. barati* *Lactobacillus* *Lactobacillus*	nd
Clostridium prevalence in OB of GE	2.9%	16.1%	4.8%

Conclusions: CD ribotype 027 was not found in any of the OBs tested. One CD ribotype 001 was detected in a gastroenteritis OB of unexplained aetiology, but several *Clostridium* spp. were found in NoV OBs. These data suggest that NoV infections enable Clostridia to colonise the gastro intestinal tracts, perhaps in a similar way as antibiotic treatment. These double infections may contribute to increased severity of the gastroenteritis episode and were revealed only by comprehensive virological and bacteriological screening of faecal samples.

Carbapenem resistance – Part 2

P1497 Waterborne infection caused by a carbapenem-resistant *Enterobacter asburiae* isolate producing the class A carbapenemase IMI-2 in France

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Objectives: Two *Enterobacter* spp. isolates were recovered from a 2-years-old child hospitalised after a drowning accident that occurred in a private pond in September 2007 in France. *Enterobacter* spp. MON-1 was isolated from a tracheal aspiration and showed a wild-type phenotype of resistance to β-lactams. *Enterobacter* spp. MON-2 was from urine and resistant to carbapenems but susceptible to ceftazidime. A peculiar synergy was noticed between clavulanic-acid and imipenem. The aim of the study was to identify the mechanism(s) responsible for carbapenem resistance in isolate MON-2.
Methods: Genomic DNA *Enterobacter* spp. isolates MON-1 and MON-2 were extracted and used as template for PCR screening of known carbapenemase genes. Plasmids were extracted using the Kieser technique. Mating-out assays were performed using *E. coli* J53 as recipient strain and a selection with amoxicillin (50 mg/L)- and azide (100 mg/L)-containing media. Random-amplified polymorphism detection (RAPD) techique was used to compare genotypically both isolates. Amplification and sequencing of 16S RNA and blaampC genes were used to identify at the genetic level the *Enterobacter* species.
Results: Molecular techniques showed that both *Enterobacter* spp. isolates belonged to the *Enterobacter asburiae* species. RAPD analysis showed that isolates MON-1 and MON-2 were clonally-related. *E. asburiae* MON-2 expressed the clavulanic-acid inhibited and carbapenem-hydrolyzing Ambler class A β-lactamase IMI-2. Mating-out assays remained unsuccessful but electroporation of a plasmid extract led to *Escherichia coli* transformant carrying a 150-kb blaIMI-2-positive plasmid that did not provide any other antibiotic co-resistance marker. Induction experiments and PCR mapping identified a LysR-type blaIMI-R2 regulatory gene upstream of the blaIMI-2 gene.
Conclusion: This study identified a class A carbapenemase of the IMI-type for the first time in Europe, after the identification of IMI-1 and IMI-2 in *Enterobacter cloacae* isolates in USA and China, respectively. This study further identifies the aquatic reservoir as a source of IMI-like producers.

P1498 Occurrence of carbapenemase-producing Enterobacteriaceae clinical isolates in the Asia-Pacific nations

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Objectives: The aim of this study was to determine the occurrence of carbapenemase (CASE)-producing Enterobacteriaceae (ENT) isolates in the Asia Pacific (APAC) region during the 2006 SENTRY Program.
Methods: ENT recovered from hospitalised patients in 37 medical centres (MC), located in 9 countries in the APAC region were tested by CLSI broth microdilution. Isolates showing imipenem and meropenem MIC $\leqslant$2 mg/L were screened for MBL- (IMP-like, VIM-like, SIM-1, GIM-1 and SPM-1) and group 2f serine Case- (KPC, IMI, SME, etc.) encoding genes by PCR and sequencing. Spectrophotometric measurement of meropenem hydrolysis was performed to access Case activity among PCR-negative isolates. Presence of quinolone (FQ) resistance (R) genes (qnr-like and aac[6′]-Ib-cr) was also accessed by PCR and sequencing. The genetic environment of Case-encoding genes was investigated by plasmid analysis, conjugation and transformation experiments.
Results: Among 844 isolates, 9 (1.1%; 1 *E. coli*, 5 *K. pneumoniae* [KPN] and 3 *P. mirabilis*) met the screening criteria. The majority of the isolates (7/9; 77.8%) was PCR-negative and did not show meropenem hydrolysis activity. One KPN isolate (#231–21D) harbouring blaKPC-2 was detected in Zhejiang, China. This isolate was recovered from a venous catheter infection in a 42 y/o male trauma victim on the 77th hospital day (HD). A second KPN (#234–49C) carrying blaIMP-4 was recovered in Wuhan (China) from the sputum (2nd HD) of a 50 d/o female infant with acute bronchopneumonia. Both cases had favorable clinical outcomes. Plasmid content of the isolate #231–21D revealed three plasmids (60-, 5- and 3.5-kb), while the transconjugant (p231–21D) strain showed only the 60-kb plasmid. The p231–21D strain showed R to β-lactams, aminoglycosides and decreased susceptibility to FQ. Further investigations detected qnrB4 in the isolate #231–21D and recipient strain p231–21D, suggesting both blaKPC-2 and qnrB4 were located in the 60-kb conjugative plasmid. Experiments failed to identify a plasmid-borne location for the blaIMP-4.
Conclusion: Occurrence of Case-encoding genes was low in the APAC region. This is the first report of coproduction of KPC-2 and QnrB4 encoded by R genes located in the same conjugative plasmid. This plasmid may further spread and escalate the R rates for their respective antimicrobial classes. IMP-4 has been detected in different countries in the APAC region, suggesting a continued dissemination of this gene in this region.

P1499 Emergence of a XDR-OXA-23-producing *Acinetobacter baumannii* clinical isolate

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Objectives: In the last years an increasing resistance of *A. baumannii* to carbapenems, mainly due to the production of OXA-type carbapenemases, has been described. Treatment options for infections with these isolates usually include aminoglycosides, polimixins and tigecycline. The aim of this work was to characterise the emergence during antibiotic therapy of an OXA-23-producing *A. baumannii* clinical isolate resistant to all available antibiotics.
Methods: *A. baumannii* HST1 and HST3 were recovered, from pus and pancreatic abscess samples, during the hospitalisation of a patient who suffered of acute necrohaemorragic pancreatitis. Meropenem therapy was administrated during 14 days before the isolation of the first strain. The second strain was isolated after the change of the therapy for an association of ampicillin and amikacin. The patient recovered after surgical procedure. Identification of the isolates was performed by 16S rRNA gene sequencing. MICs for the β-lactamic antibiotics and to amikacin were determined by the Etest method (AB Biodisk, Solna, Sweden) and VITEK 2 system. The susceptibility of the isolates to colistin, minocyclin and tigecycline was determined by the agar dilution method according to CLSI guidelines. Susceptibility to gentamicin, kanamycin, netilmicin and ciprofloxacin was tested by disk diffusion method. Oxacilinase genes (OXA-23, 40, 51, and 58) were sought by a multiplex PCR. ISAba1 insertion sequence upstream of blaOXA-23 gene was also searched by PCR. PFGE (ApaI) was performed for comparison of these isolates with imipenem-resistant clone that persist in Portugal since 1998.
Results: The strains were resistant to all tested antibiotics, including colistin, minocyclin and tigecycline (MICs were 32, 64 and 16 mg/L, respectively). HST1 was susceptible to amikacin; however, after a therapy change to amikacin plus ampicillin, a second isolate was collected demonstrating resistance to amikacin.
PCR reactions detected the presence of blaOXA-23 (confirmed by sequencing reactions) and ISAba1upstream of the OXA-23 gene.
PFGE showed that the isolates' profile was related with the imipenem-resistant *A. baumannii* clones disseminated throughout the country, including the endemic OXA-40 producing *A. baumannii* clone.

Conclusions: This is a first description of a XDR-*A. baumannii* that evolved during antibiotic therapy, from a well disseminated clone. The spread of this isolate is a worrisome situation, especially for debilitated patients.

P1500 Molecular characterisation of carbapenem-resistant *Acinetobacter* species in an Irish tertiary referral hospital

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Objective: Carbapenem resistance is increasing among *Acinetobacter* species worldwide with important therapeutic and infection control implications with this developing trend. There is little data regarding carbapenem resistance in *Acinetobacter* in Ireland. We aim to characterise the carbapenem resistance mechanisms of *Acinetobacter* isolates in our hospital.
Methods: Non-consecutive *Acinetobacter* isolates were prospectively collected from May 2005 to October 2007. Speciation was confirmed by rpoB sequencing. Carbapenem-resistant isolates were tested phenotypically for metallo-β-lactamase using the MBL E-test while genes encoding for Ambler class B (VIM, IMP) and class D (OXA-23 group, OXA-24 group, OXA-51 group and OXA-58) enzymes and insertion sequence ISAba1 were sought using PCR, gel electrophoresis and nucleotide sequencing. Isolates of the same species were typed using pulsed field gel electrophoresis (PFGE).
Results: 114 *Acinetobacter* isolates were collected over the 30-month period. 12 isolates were resistant to meropenem. 10 were *Acinetobacter* genospecies 3 (AG3), 2 were *Acinetobacter baumannii* and *Acinetobacter johnsonii* respectively. All isolates had tigecycline and colistin MICs of $\leqslant 0.5$ mg/L. Although the isolates tested positive phenotypically for MBL, subsequent PCR for VIM or IMP did not reveal any corresponding amplicons. Instead, they were positive for the OXA-23 group gene and ISAba1 insertion sequence was also present immediately upstream of the gene in all the isolates. Subsequent nucleotide sequencing confirmed the OXA gene to be blaOXA-23 in all 12 isolates. PFGE also revealed the polyclonal origins of the AG3 isolates.
Discussion: Carbapenem-resistant isolates have emerged in a significant proportion (10%) of *Acinetobacter* spp. in our hospital. The epidemiology is unusual with the predominance of *Acinetobacter* genospecies 3 instead of *A. baumannii* as with most studies. To the authors' knowledge, this is also the first documented clinical isolate of carbapenem-resistant *A. johnsonii* in the literature. The study also demonstrated that OXA-23 is the predominant gene for carbapenem resistance in our isolates and ISAba1 probably plays a pivotal role in regulating its expression. The emergence of carbapenem-resistant *Acinetobacter* isolates reiterates the need for stringent infection control policies, judicious use of antimicrobial agents, resistance surveillance and research into new anti-Gram-negative antibiotics.

P1501 *Acinetobacter baumannii* infections in thermally injured: a multidrug-resistant blaOXA-23 outbreak in a burn wound centre, Brussels

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Objectives: Admission screening of a patient transferred from Algeria revealed colonisation by a MDR carbapenem-resistant *Acinetobacter baumannii* (Ab) strain. We aimed to study the epidemicity of this MDR-Ab and evaluate the efficacy of infection control measures for controlling the outbreak.
Methods: Clinical isolates (57) from the 8 patients and the environment (19) were collected and analysed molecularly by AFLP, PFGE fingerprinting, PCR/sequencing targeting several β-lactamase genes including OXA-carbapenemases. Strict infection control measures (closure of unit, enforcement of contact precautions, terminal room disinfection with hydrogen peroxide ultra mist, increased hospital educational efforts, increased screening, including personnel and environment) were taken.
Results: Overall 8 patients where infected on wounds and there respiratory tract during this 11 month period. Four patients died. These critically ill had all 3rd degree burns (mean Total Body Surface Area (TBSA) 38%), a mean age of 62 y with significant underlying co-morbidities and co-infections with other pathogens. The direct cause of death was often sepsis, but no Ab was isolated from the blood. AFLP genotypic analysis showed the presence of 3 different clones in the index case. Subsequent molecular typing revealed the emergence of one single epidemic clone, which retained the MDR carbapenem-R phenotype (OXA-23 class D carbapenemase). This epidemic clone evolved into a closely related variant that persisted for around 8 months in the unit. All patients were treated by Polymixin E the only active agent against this isolate. One out of the 4 survivors was cured and no major side effects were observed. The implementation of strict infection control measures allowed to terminate the epidemic spread.
Conclusions: Despite systematic screening on admission this imported outbreak caused by a MDR carbapenem-resistant OXA-23 producing Ab illustrates the epidemic potential of this difficult-to-treat nosocomial pathogen and the need of permanent alertness on hospital infection control as well as the usefulness of molecular typing methods to delineate the epidemiologic relationship of the isolates.

P1502 Dissemination of carbapenem-resistant *Acinetobacter baumannii* producing the OXA-23 carbapenemase in a university hospital, Tunisia

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Objective: To characterise the clonal relationship, the β-lactamase content and genetic support of carbapenem-resistant *Acinetobacter baumannii* isolates recovered in the University Hospital Sahloul in Tunisia during a two-years period.
Methods: Ninety-nine non-repetitive carbapenem-resistant *A. baumannii* isolates were recovered from January 2005 to December 2006 from urine, pus and blood samples. The presence of oxacillinase or metallo-β-lactamase genes was detected by antibiotic susceptibility testing analysis, PCR and sequencing. In particular, genes coding for the carbapenem-hydrolysing oxacillinases (CHDL) OXA-23, OXA-40 and OXA-58 subgroups were searched. Genotyping was done by pulsed field gel electrophoresis (PFGE) using ApaI endonuclease.
Results: Most *A. baumannii* isolates were from patients hospitalised in intensive care units and the urology ward. Antibiotyping allowed to divide these isolates into two main groups (A and B), both being resistant to all β-lactams including carbapenems. The thirteen *A. baumannii* isolates belonging to group A possessed the blaOXA-23 gene. This gene was very likely chromosomally-located. Insertion sequence ISAba1 was identified upstream of the blaOXA-23 gene, enhancing its expression by providing strong promoter sequences. *A. baumannii* isolates belonging to clone B did not express any carbapenemase activity and were found negative for CHDL encoding genes.
Conclusion: This study reports on the first identification of β-lactamase OXA-23 in *A. baumannii* in Tunisia. The gene encoding OXA-23 was located on the chromosome of clonally-related *A. baumannii* isolates being the source of an outbreak. Interestingly, this epidemiological survey revealed the concomitant dissemination of two different carbapenem-resistant *A. baumannii* clones in the same hospital during the same period of time.

P1503 Clonal spread of carbapenem-resistant *Acinetobacter baumannii* possessing blaOXA-23 in a Bulgarian hospital

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Objectives: To investigate the mechanism of carbapenem resistance (CR) in clinical isolates of *Acinetobacter baumannii*, collected during

the period 1999–2006 from the University Hospital in Pleven, Bulgaria and to evaluate the clonal relationship between the isolates.
Methods: From October 1999 to September 2006, 29 CR and 15 carbapenem susceptible (CS) *A. baumannii* isolates, collected from different wards, were studied. Identification was confirmed by gyrB multiplex PCR and ARDRA. Antimicrobial susceptibility was tested by VITEK 2 and agar dilution method and the results were interpreted according to the current CLSI guidelines. Electrotransformation experiments were performed to determine carbapenem resistance as chromosomal- or plasmid-mediated. Detection of the carbapenemase encoding genes and the associated insertion sequences was performed by PCR, followed by sequence analysis. Isolates were genotyped by PFGE.
Results: All studied isolates revealed a multidrug resistance phenotype and possessed blaOXA-51-like and blaAmpC genes. In addition, a blaOXA-23-like gene was detected in all CR isolates. ISAba1 was located upstream of the blaOXA-23-like gene. Sequence analysis confirmed the presence of blaOXA-23. The unsuccessful electrotransformation experiments suggested chromosomal location of the blaOXA-23 gene. The CR isolates exhibited 2 PFGE pulsotypes. Pulsotype B dominated in the ICU between 1999 and 2000 and was also detected in three CS strains isolated from ICU patients during this period. Pulsotype A accounted for 24 CR strains and was spread among several wards during the period 2003–2006, but dominated in the ICU. However, one pulsotype A strain was already recovered in 1999.
Conclusions: The blaOXA-23 gene and ISAba1 were associated with carbapenem resistance. The increased rate of isolation of CR *A. baumannii* in the hospital during the period 1999–2006 was due to intrahospital dissemination of epidemic strains belonging to 2 clonal groups, A and B. These data demonstrate both the epidemic spread of CR *A. baumannii* and its longevity in the hospital environment.

P1504 OXA-23 and OXA-58 β-lactamase producing *Acinetobacter baumannii* clinical isolates from Bulgarian hospitals

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Objectives: To investigate the mechanism of carbapenem resistance (CR) in clinical isolates of *Acinetobacter baumannii* collected during 2005–2006 from 2 University Hospitals in Bulgaria and to evaluate the clonal relationship between them.
Methods: A total of 80 nonduplicate isolates of *A. baumannii* collected from different wards in Sofia (UHS) and Varna (UHV) were studied. Species identification was confirmed by gyrB multiplex PCR and ARDRA. Antimicrobial susceptibility was tested by VITEK 2 and agar dilution and the results interpreted according to the current CLSI guidelines. Electrotransformation experiments were performed to define CR as chromosomal or plasmid-mediated. Detection of carbapenemase encoding genes and associated insertion sequences was performed by PCR, followed by sequence analysis. Isolates were genotyped by PFGE.
Results: The results are summarised in the table. Twelve CR strains were isolated, all exhibiting a multidrug resistance phenotype. blaOXA-23-like gene was found in 8 CR isolates and blaOXA-58-like gene was detected in 4 CR and 1 carbapenem susceptible (CS) isolates. All 80 isolates were positive for blaOXA-51-like gene. Sequence analysis confirmed the presence of blaOXA-23 and blaOXA-58. There was no difference in the nucleotide sequence of the blaOXA-58 genes from UHV. CR was transferable with the blaOXA-58 gene and identified to be plasmid-located. The blaOXA-23 gene was not transferable. Eleven PFGE pulsotypes were detected among all studied isolates. The CR strains exhibited 3 PFGE pulsotypes. Pulsotype A was isolated from Medical ICU and Surgical ICU in UHS and pulsotype B from the Surgical ICU of the same hospital. Pulsotype D is represented by 2 OXA-58 positive strains, recovered from two wards in UHV. Pulsotypes A and D were also found in CS strains.
Conclusions: CR from UHS was associated with the spread of 2 distinct clones. Those with pulsotype A have acquired either an OXA-23 or OXA-58 carbapenemase. The natural reservoirs of these genes remain unknown. In the second hospital, UHV, 2 isolates were OXA-58 positive however, only one was CR. Molecular typing reveals intra-hospital, but not inter-hospital dissemination of CR *A. baumannii* and demonstrates that the emergence of CR *A. baumannii* is caused by acquisition of resistance determinants by previously susceptible strains and subsequent cross transmission.

Hospital	No. isolates (CS/CR)	ISAbal-OXA	ISAba3-OXA	Pulsotype (No.)
UHS	2/11			
OXA-23	0/8	8	negative	A(5), B(3)
OXA-58	0/3	negative	3	A(3)
OXA-negative	2/0	–	–	A(1), C(1)
UW	66/1			
OXA-58	1/1	2	2	D(2)
OXA-negative	65/0	–	–	D(21), E(25), F(1), G(5), H(1), I(9), J(1), K(2)

P1505 Assessment of carbapenem resistance and presence of acquired carbapenemases in Russian nosocomial isolates of *Acinetobacter* spp.

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Objectives: To determine resistance rates to imipenem (IPM) and meropenem (MER) and occurrence of acquired metallo-β-lactamases (MBLs) and class D (OXA) carbapenemases in nosocomial *Acinetobacter* spp. from Russian ICUs.
Materials and Methods: A total of 464 consecutive nosocomial isolates collected as part of the national surveillance study RESORT in 30 ICUs of 20 Russian cities in 2002–2004 were studied. The susceptibilities to IPM and MER were determined by agar dilution method and interpreted according to CLSI guidelines. A double-disk synergy test with EDTA was used for phenotypic detection of MBL production. The presence of the genes for VIM- and IMP-type MBLs and the genes for acquired class D carbapenemases of three groups: OXA-23-like, OXA-40-like and OXA-58, was examined by PCR.
Results: A total of 11(2.4%) and 17(3.7%) were nonsusceptible to IPM (MIC range: 8–128 μg/ml) and MER (MIC range: 8–64 μg/ml), respectively. MBL production was not detected among carbapenem-nonsusceptible isolates by either phenotypic or molecular tests. The genes for acquired OXA-type carbapenemases were found in 11 (2.4%) isolates of *A. baumannii* exhibiting variable MICs of IPM (4–32 μg/ml) and MER (8–32 μg/ml). Eight of these isolates from two hospitals of Moscow and Novosibirsk harboured the genes for OXA-58 and the remaining three isolates from Moscow and Irkutsk carried the genes for OXA-23-like enzymes. No acquired carbapenemase genes were detected in the other six isolates exhibiting increased resistance to IPM or MER (MICs: 8–128 μg/ml).
Conclusions: Resistance to carbapenems remains rare among nosocomial Acinetobacters in Russia. However, the identification of acquired OXA-type carbapenemases in isolates from geographically distant regions of Russia poses the risk of future dissemination of resistance.

P1506 The dissemination of OXA-23 harbouring VIM-2 among *Acinetobacter baumannii* isolates in two major Saudi Arabia hospitals

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Objectives: Carbapenems have become the drugs of choice for serious *Acinetobacter baumannii* infections, however, they are being compromised by the emergence of carbapenem-hydrolysing β-lactamases of molecular class B and D. The IMP and VIM are more prevalent in the Far East, however, OXA carbapenemases are more reported in Europe. In addition, the genes encoding OXA-23-like and OXA-51-like enzymes have been found to be linked to insertion element ISAba-1,

with the OXA-58-like encoding gene adjacent to ISAba-2, ISAba-3 and IS18. The aim of this work was to investigate the dissemination of these β-lactamases and to reveal if they are spread clonally.

Methods: A total of 28 nonrepetitive, *A. baumannii* strains collected between January 2006 and April 2007 from different specimens. Isolates were identified to the species level by restriction analysis of the 16S-23S rRNA intergenic spacer sequences. The (MIC) of antibiotics was determined by the agar dilution method following the BSAC Guidelines. The potency of tigecycline was tested by Disk diffusion test. The metallo-β-lactamases and the IS genes were detected by multiplex polymerase chain reaction (MPCR) and PCR respectively, and their identities were confirmed by sequencing. Pulsed-field gel electrophoresis (PFGE) typing was performed using ApaI restriction endonuclease.

Results: Out of 28 isolates, 11 were resistant to imipenem (MIC > 8 mg/L), of which 4 were highly resistant (MIC = 64 mg/L). Most of these isolates were also multi-drug resistant, they were resistant to meropenem, ciprofloxacin, gentamicin and many β-lactam antibiotics, but were all sensitive to tigecycline. Six imipenem-resistant isolates (MIC 16–64 mg/L) had OXA-23, of which 4 contained ISAba-1 and ISAba-2 and two with only ISAba-1. Only one isolate (MIC 16 mg/L) had OXA-24 and ISAba-1. One imipenem-sensitive isolate (MIC 0.25 mg/L) harboured ISAba-2, ISAba-3 and IS18. A mixture of 21 imipenem-sensitive and imipenem-resistant isolates had ISAba-1 upstream of blaOXA-51 gene. All imipenem-resistant isolates were positive by PCR for blaVIM gene, and sequencing confirmed that the PCR products were 100% identical to the VIM-2. The PFGE showed that, in one hospital, 4 imipenem-resistant isolates clustered in one big group. The rest were either clustered in two or grouped with imipenem sensitive isolates.

Conclusion: Both Oxacillinases and metallo-lactamase, are now prevalent in imipenem-resistant *A. baumannii* in Saudi Arabia hospitals.

P1507 Molecular investigation of sequential isolates of multidrug-resistant *Acinetobacter baumannii* clones producing the OXA-40 carbapenemase

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Objective: The aim of the study was to analyse sequential isolates of two multi-drug-resistant *A. baumannii* clones producing the blaOXA-40-like carbapenemase gene, including molecular and sequence based typing based on the ompA (outer membrane protein A), csuE (part of a pilus assembly system, essential for biofilm formation) and blaOXA-51-like (the intrinsic carbapenemase gene in *A. baumannii*) genes.

Material and Methods: 15 isolates were selected from a collection of total isolates of *A. baumannii* obtained from a Hospital in Northern Spain during the years 1999, 2002 and 2005. The representatives were three per year and clone including blaOXA-40 positive and negative isolates. Clonal relatedness was confirmed by PFGE with Apa I enzyme. Multiplex PCR designed to selectively amplify the ompA, csuE and blaOXA-51-like alleles of a previously identified clonal complex designated Group 1 was carried out on all isolates, and sequencing of these genes carried out on representative isolates. To locate the blaOXA-40 carbapenemase gene plasmid DNA was digested with EcoRI, PstI and HindIII endonucleases and hybridised with an OXA-40 specific probe to locate the corresponding gene.

Results: Sequence typing confirmed that clones I and II belong to two highly distinct lineages and comparison with clones from the United Kingdom showed its homology with sequence type groups 3 and 1 respectively. All isolates were PCR positive for blaOXA-51-like, confirming their identity as *A. baumannii*. No isolates were positive for bla OXA-23–like. Plasmids of similar sizes (ranging from 2.5 to 125 kb) were observed in all isolates, frequently combined in groups of four structures. Using a blaOXA-40 specific probe we obtained signal on different structures but most frequently on a plasmid of 32 kb.

Conclusion: The OXA-40 carbapenemase gene is present in plasmids harboured by different genotypes indicating the relatioship with a mobile structure. Both clones bore plasmids of similar sizes frequently associated in groups of four structures per isolate.

P1508 Identification of an OXA-58 carbapenemase in an unusual Portuguese *Acinetobacter baumannii* clinical isolate

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Objectives: Several carbapenem oxacillinases have been reported recently in *A. baumannii*. Our group characterised a multidrug resistant (MDR) clone producing an OXA-40 carbapenemase endemic in Portugal and Spain. Recently, imipenem-resistant isolates with slight different antibiogram has been found in a Central Hospital Santo Antonio dos Capuchos (HSAC) at Lisboa. The aim of this study was to screen for carbapenemases in *A. baumannii* imipenem-resistant isolates.

Methods: The isolates were grouped on basis of susceptibility profiles determined by VITEK 2 System® and RAPD profiles. Amplification was performed using specific primers for OXA type enzymes and class 1 integrons. Plasmid extraction was prepared by Kado & Liu method.

Results: Fifteen representative isolates were clustered in 4 groups: A1 (n = 3) susceptible to tobramycin and colistin; A2 (n = 3) susceptible to aminoglycosides (gentamicine, amikacin, tobramycin and netilmicin), colistin, tetracycline, intermediate/resistant to ceftazidime and levofloxacin; A3 (n = 3) susceptible to aminoglicosydes and colistin but intermediate to tetracycline and resistant to ceftazidime; and group A4 (n = 3) with variable susceptibility. OXA-40 enzyme was found in 10 isolates, not correlating with any particular group. The Ac 156976 isolate, included in group A4, showed an OXA-58 enzyme and was resistant to aminoglycosides, ceftazidime, and imipenem; susceptible to ciprofloxacin, intermediate to meropenem. The plasmidic blaOXA-58 gene was part of a class 1 integron gene cassette.

Conclusion: The study report the identification of an OXA-58 enzyme in Portugal for the first time, and highlight the possibility of an emergence of a new multidrug resistant clone and its dissemination.

P1509 Nosocomial dissemination of carbapenem-resistant *Acinetobacter* spp. isolates in a general hospital in Thessaloniki, Greece

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Objectives: To characterise the clonal relationship and the β-lactamase content of carbapenem-resistant *Acinetobacter* spp. isolates recovered in a University Hospital in Thessaloniki, Greece.

Methods: Twenty-one non-repetitive carbapenem-resistant *Acinetobacter* spp. isolates were recovered from August 2001 to November 2006 period at the Papageorgiou General Hospital, Thessaloniki, Greece. Species identification was determined by the biochemical API32 GN test (bio-Mérieux, France) and by 16S rRNA sequencing. Susceptibility testing was done by disk diffusion method and Minimum Inhibitory Concentration (MICs) were determined by agar dilution. Genes coding for the carbapenem-hydrolyzing class D β-lactamases OXA-23, OXA-40, OXA-58 and OXA-51 subgroups and for VIM- and IMP-type metallo-β-lactamases (MBLs) were searched. The isolates were also screened for the presence of ISAba1 in the promoter region of the blaOXA-51-like gene in order to investigate the role of ISAba1 in blaOXA-51 expression. Genotyping was done by pulsed field gel electrophoresis (PFGE) after digestion by ApaI.

Results: All isolates were identified as *A. baumannii* except one isolate identified as *Acinetobacter* genomospecies 16. Genotyping revealed that the twenty *A. baumannii* isolates belonged to five different pulsed-field gel electrophoresis clones. Clones 1 to 3 including sixteen *A. baumannii* isolates harboured the blaOXA-58 gene whereas clones 4 and 5 including four isolates did not possess any carbapenemase gene. ISAba1 was identified upstream of the blaOXA-51 gene in three blaOXA-58 negative *A. baumannii* isolates. In one of these isolates, ISAba1 was truncated immediately upstream of the blaOXA-51 gene by a novel insertion sequence named ISAba9 that may provide promoter sequences for

blaOXA-51 expression. *Acinetobacter* genomospecies 16 harboured VIM-4 MBL determinant. The blaOXA-58 gene was most often plasmid-located.

Conclusion: This study highlights the diversity of the mechanisms involved in carbapenem resistance in an *Acinetobacter* spp. Furthermore, this is the first description of the MBL VIM-4 determinant in *Acinetobacter* spp. and the first description of ISAba9, a novel insertion sequence providing promoters for oxacillinase genes expression.

P1510 Persistence of pan-resistant *Acinetobacter baumannii* strains recovered from blood cultures in an intensive care unit

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Objectives: The aim of our study was to investigate the resistance mechanisms to carbapenems and to characterise the genotypes of panresistant *A. baumannii* (Ab) strains, isolated from blood cultures of Intensive Care Unit's (ICU) patients, in a tertiary hospital in Greece, during eight months period (9/2006–5/2007)

Methods: During the study period 22 panresistant Ab strains were recovered from blood cultures of ICU's patients. The strains were identified by VITEK 2 automated system (bioMerieux). Antibiotic susceptibility was tested by disk diffusion method and MICs were determined by VITEK 2. All strains were investigated for the production of metallo-β-lactamases (MBL) by (a) an in house EDTA synergy test with imipenem and EDTA discs (EDTA/DDST) and (b) E-test strips of imipenem and imipenem/EDTA (MBL E-test). The presence of the bla genes of the VIM, OXA-23, OXA-24, OXA-51 and OXA-58 types were identified by PCR assays. All strains were genotyped by pulse field gel electrophoresis (PFGE) after digestion with ApaI.

Results: All of the examined strains were unanimously resistant to all β-lactams, including carbapenems, quinolones and aminoglycosides and were sensitive to colistin. None of the strains revealed MBL production by EDTA/DDST, while 9/22 (41%) showed MBL production by MBL E-test. The blaVIM gene was not detected in any of the strains by PCR. All strains presented blaOXA-51 and blaOXA-58 type genes by PCR. Molecular analysis of 21 Ab strains by PFGE showed one major clone (clone A) with two subtypes (A1 and A3) and the remaining strain exhibited a unique pattern (U).

Conclusions:

1. Persistence of panresistant Ab strains, susceptible only to colistin, from blood cultures of patients in ICU was observed during eight months period
2. All strains produced oxacillinases of the OXA-51 and OXA-58 types
3. The majority of our strains belonged to a single clone, which in combination with their panresistance, indicates the need for more strict reinforced infection control in ICU

P1511 Genetics and expression of the carbapenem-hydrolyzing oxacillinase genes blaOXA-51 and blaOXA-58 in *Acinetobacter baumannii* isolates from blood cultures in a university hospital in Athens, Greece

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Objectives: Molecular studies showed that carbapenem-resistant *Acinetobacter baumannii* strains isolated from blood cultures of hospitalised patients in the University General Hospital "Attikon", belonging to several clones, contain both blaOXA-58 and the naturally occurring blaOXA-51-like genes. The objective of our study was to examine the genetic structures surrounding the blaOXA-51 and blaOXA-58 oxacillinase genes.

Methods: A total of 27 imipenem-resistant (harbouring blaOXA-58 and blaOXA-51-like genes) isolates collected from blood cultures of hospitalised patients in the University General Hospital "Attikon", were studied. Analysis of the sequences bracketing the blaOXA-51 and blaOXA-58 genes were evaluated by PCR mapping using combinations of the ISAba primers and the blaOXA-51-like and blaOXA-58-like reverse primers (Poirel and Nordmann, 2006). Bacterial clones were identified by PFGE with ApaI.

Results: ISAba1 element was identified upstream of the blaOXA-51 gene in all clones of *A. baumannii* tested. Some variability of the promoter sequences identified upstream of the blaOXA-58 gene among the analysed isolates was indicated by our results. In all isolates an ISAba3 element was identified upstream of the blaOXA-58 gene but in some cases it was truncated by the insertion of ISAba2. Additionally the ISAba3 was present (not always at the same distance) upstream of the start codon of blaOXA-58. Another interesting feature was the presence of ISAba3 downstream of blaOXA-58.

Conclusions: This work identified variable genetic structures at the origin of acquisition and expression of a carbapenem-hydrolyzing β-lactamase OXA-58 harboured by non-clonally related *A. baumannii* isolates.

P1512 Presence of the carbapenem-hydrolyzing oxacillinase Oxa-58 in an *Acinetobacter* phenon 6/ct13TU clinical isolate

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Objectives: The main nosocomial pathogen within the *Acinetobacter* genus is *Acinetobacter baumannii*. Carbapenems usually retain good activity against these microorganisms. In spite of this, carbapenem resistance in *A. baumannii* has been reported worldwide. The main aim of our study was to investigate the mechanism of resistance to imipenem (IP) in an *Acinetobacter* phenon 6/ct13TU clinical isolate.

Methods: The study was performed in two *Acinetobacter* spp. (Ac054 and Ac058) isolates collected from two Spanish hospital during the same period of time. The characterisation of these strains was done by ARDRA and the MICs of IP were determined by Etest. The detection of OXA-58 was done by PCR and sequencing with specific primers from *A. baumannii*. Expression of the OXA-58 gene was determined by RT-PCR using the specific primers for this gene and the 16S as internal control. The genetic location of the gene was investigated by using the genomic mapping with I-CeuI followed by Southern blot and double hybridisation with probes for the blaOXA-58 gene and for the 23S gene.

Results: One isolate was identified as *A. baumannii* (Ac058) and the other as *Acinetobacter* phenon 6/ct13TU (Ac054). Both isolates, as expected, were different by Pulse-Field Gel Electrophoresis. Presence of the OXA-58 gene was determined by PCR with specific primers. The OXA-58 genes were sequenced and presented 100% homology. Analysis of gene expression by RT-PCR also revealed that OXA-58 was expressed in both isolates. However, *A. baumannii* strain Ac058 had a MIC of IP >32 mg/L while *Acinetobacter* phenon 6/ct13TU strain Ac054 had a MIC of IP = 6 mg/L which implies the presence in *A. baumannii* of an additional mechanism of resistance to imipenem.

Conclusion: The OXA-58 gene, with 100% homology to the same gene found in *A. baumannii*, has been detected for the first time in an *Acinetobacter* phenon 6/ct13TU clinical isolate. Its location in a plasmid suggests that these resistance genes may be transferred from one species to another.

P1513 Outbreak of carbapenem-resistant *Acinetobacter baumannii* producing the carbapenemase OXA-58 in Turkey

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Objectives: The use of carbapenems to eradicate multi-drug resistant (MDR) *Acinetobacter baumannii* (Ab) may become compromised by the spread of carbapenem-resistant genes. Carbapenem-hydrolyzing class D β-lactamases (OXA-23, OXA-24, OXA-40, or OXA-58) producing Ab isolates have been reported worldwide. Here we describe the first outbreak of OXA-58 producing Ab in Turkey.

Methods: All MDR clinical isolates of Ab, recovered between June and November 2003 in a tertiary-care hospital in Antalya, were

analysed for their DNA content (genomic or plasmid), by isoelectric focusing (IEF), conjugation assays and PFGE. PCR were performed for blaIMP, blaVIM, blaOXA-23-like, blaOXA-40-like, blaOXA-58 genes and blaOXA-51-like genes, blaTEM gene, blaOXA-20, the chromosomal class C β-lactamase blaAMPC gene and the presence of ISAbaI inserted upstream of the β-lactamase genes. β-lactamase expression was monitored by hydrolysis and iso-electrofocusing.
Results: 116 MDR *A. baumannii* isolates were isolated from 23 patients, mostly from respiratory samples and from 10 environmental samples. These MDR isolates, which belonged to a single clone, remained susceptible to colistin and rifampin only and produced the carbapenem-hydrolyzing oxacillinase OXA-58. In addition, they were also positive for the blaOXA-51, for a point mutant derivative of blaAMPC (ADC-7) genes and for ISAba1. One isogenic carbapenem-susceptible strain lost its blaOXA-58 gene, through loss of its plasmid. IEF confirmed the expression of β-lactamases OXA-58 and AmpC. The blaOXA-58 gene was located onto a non self-transferable 50-kb plasmid that could be electroporated to Ab7010 reference strain and conferred a β-lactam resistance pattern consistent with the expression of OXA-58 carbapenemase. PCR mapping and sequencing identified similar genetic structures surrounding the blaOXA-58 gene as those reported adjacent to the blaOXA-58 prototype gene, e.g. presence of two insertion sequences ISAba3-like elements bracketing blaOXA-58 gene.
Conclusion: This is the first molecular description of an outbreak of OXA-58-producing Ab isolates in Turkey, further underlining the global spread of such carbapenemase-producing strains.

P1514 Acquired carbapenemase-encoding genes among Gram-negative isolates in USA medical centres: report from the MYSTIC Program (2007)

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Objective: To evaluate the occurrence of acquired carbapenemase-encoding genes among Gram-negative patient isolates recovered from USA MC in the 2007 MYSTIC Program.
Methods: 1,392 Enterobacteriaceae (ENT), 133 *Acinetobacter* spp. (ASP) and 465 *Pseudomonas* spp. (PSP) clinical isolates from 15 USA MC were susceptibility tested by CLSI reference broth microdilution method. ENT and PSP isolates showing MIC values ⩽2 mg/L and ⩽16 mg/L, respectively, for imipenem and meropenem were screened for KPC- and metallo-β-lactamase (MBL)-encoding genes. ASP isolates showing MIC values ⩽16 mg/L for imipenem and meropenem were screened for MBL- and OXA-encoding genes. Presence of ISAba1 upstream of the blaOXA-51 and clonality were also investigated by PCR and PFGE, respectively. Additionally, ASP isolates from previous MYSTIC Program years were tested for OXA genes.
Results: 42 (3.0% of the total) ENT isolates were screened and 35 (2.5%) isolates from MC located in New York (24/35; 68.6%), New Jersey (10/35; 28.6%) and Ohio (1/35; 2.8%) harboured blaKPC. This gene was found in *C. freundii* (2), *E. coli* (1), *E. cloacae* (3), *K. oxytoca* (1) and *K. pneumoniae* (28). Among 133 ASP, 50 (37.6%) isolates were screened and blaOXA-23 was detected in 10 isolates from MC in New York (3), Kentucky (3), Arkansas (2) and Colorado (2), while blaOXA-24 was detected in 5 isolates from New Jersey. ISAba1 was associated with blaOXA-51 in 76.5% of the cases and clonal dissemination of blaOXA-carrying ASP within MC was noted. In the retrospective sample of ASP collected from 1999 to 2006, 42 (6.8%) isolates were screened for blaOXA. blaOXA-23 was observed in 2 isolates from Tennessee (2004) and one isolate each from Kentucky and Washington (2005). Among the 36 (7.7%) PSP isolates screened, no carbapenemases were detected. MBL-encoding genes were not observed.
Conclusions: Although the results presented here document an alarming presence of blaKPC, the rate (2.5%) was lower when compared with the previous MYSTIC year (4.5%; P = 0.0063; OR = 1.80, CI 95% 1.15–2.82). However, the KPC gene has spread among several different ENT species, while it was detected only in *Klebsiella* spp. in the previous year. blaOXA genes have been detected in several MYSTIC Program MC within the USA; first detected in 2004. blaOXA genes were only observed in consecutive years in Kentucky, suggesting a sporadic appearance in ASP, overall. MBL-encoding genes are still quite rare in the MYSTIC Program.

P1515 Characterisation of resistance genes among *Acinetobacter* spp. isolates in Singapore hospitals

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Objectives: Carbapenem-resistant *Acinetobacter baumannii* are increasing causes of nosocomial infection worldwide. Several mechanisms are responsible for carbapenem resistance in *Acinetobacter* spp. The carbapenemases described so far belong to either the OXA class D family of serine β-lactamases or the IMP/VIM class B family of metallo-β-lactamases. The aim of our study was to investigate the possible mechanism of resistance to imipenem in *Acinetobacter* spp. clinical isolates with regards to the OXA classes and metallo-β-lactamases.
Methods: We studied 145 isolates of *Acinetobacter* spp. collected over 2 months in 2006 from all public hospitals in Singapore. Susceptibility patterns according to individual laboratories were correlated with the resistance genes present. Isolates was screened for blaOXA-23-like, blaOXA-24-like, blaOXA-58-like, and blaOXA-51-like genes using a multiplex PCR. Putative metallo-β-lactamase genes were amplified from the collection by using published degenerate primers.
Results: 58 strains were carbapenem (imipenem/meropenem) susceptible while 87 strains were carbapenem resistant. Among carbapenem resistant strains, 86% and 85% carried blaOXA-23-like and blaOXA-51-like respectively. Carbapenem-susceptible strains carried these genes at a frequency of 6% and 62% respectively. Five of both the carbapenem resistant and susceptible strains carried the blaOXA-58-like gene. Interestingly, among carbapenem-susceptible strains, 43% carried gene products coding an blaIMP-like gene. Among carbapenem resistant strains, the frequency was 50%.
Conclusion: The presence of blaOXA-51 did not correlate with the level of carbapenem resistance. The presence of blaOXA-23-like genes did predict for imipenem resistance. Apparently imipenem susceptible strains may carry the blaIMP-like gene.

P1516 The mechanism of carbapenem resistance in *Acinetobacter baumannii* clinical isolates and expression of OXA-72 in *Pichia pastoris*

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Objectives: To elucidate the mechanism of carbapenams resistance in *Acinetobacter baumannii* (*Acinetobacter* spp.) and to express OXA-72 in *Pichia pastoris*.
Methods: In 2004, we collected 96 *Acinetobacter* spp. isolates from sputum of hospital-acquired pneumonia patients at 2 hospitals in Jinan, China. Of these, 5(5.2%) were identified to be carbapenem-resistant by E-test. Firstly, we investigated the carbapenems genetype of these 5 isolates by polymerase chain reaction (PCR) with the specific primers of bla(OXA-23), bla(OXA-24), bla(IMP) and bla(VIM). The PCR products were then sequenced. Secondly, we analysed the profiles of out membrane proteins of the 5 isolates. Out membrane proteins were prepared by supersound, collected by ultra-centrifugation and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Thirdly, to express the carbapenemase OXA-72 in *Pichia pastoris*, we amplified oxa-72 gene by PCR using the extracted genomic DNA of the isolate 40 as a template. After the amplified gene was cloned into vector pGAPZalphaA, linearised recombinant plasmid oxa72-pGAPZalphaA was transformed to *Pichia pastoris* GS115 by electroporation. Then the positive transformants were screened by colony PCR, western blot analysis and Nitrocefin test.
Results: PCR with the presence of bla(OXA-23)-specific primers amplified a circa 1083-bp fragment with three isolates(isolate 11, 66 and 67), their DNA sequences were identical with blaOXA-23. One isolate (isolate 40) obtained a circa 1043bp PCR product with the presence

of bla(OXA-24)-specific primers, the DNA sequence was identical with blaOXA-72(GenBank accession numbers AY739646). A protein of 29kDa decreased in one carbapenem-resistant isolate (isolate 66) and a protein of 24kDa overexpressed in two carbapenem-resistance isolates (isolate 66 and 67). One positive transformant was obtained with PCR, western blot and Nitrocefin test all positive.
Conclusion: These findings indicates that producing class D β-lactamase OXA-23 and OXA-72 was one of the mechanism for carbapenems resistance of *Acinetobacter* spp. in Jinan. The decrease of a 29 kDa out membrane protein and the overexpression of the 24kDa penicillin-binding protein were also related to the carbapenems resistance to in *Acinetobacter* spp. We found carbapenemase OXA-72 which is seldom reported and successfully expressed it in *Pichia pastoris*. Now we are purifying the carbapenemase OXA-72 and do further study on the properties of it.

P1517 Warning! Oxacillinase-mediated carbapenem resistance spreading in Enterobacteriaceae

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Introduction: Resistance to expanded-spectrum cephalosporins in Enterobacteriaceae has been reported to be related to the production of Ambler class A extended-spectrum β-lactamases (ESBLs) and chromosomal and plasmid-encoded AmpC cephalosporinases. However, increasing number of reports regarding β-lactamase mediated resistance to carbapenems in Enterobacteriaceae are being published.
Objectives: Between 2002–2006, we identified 21 Enterobacteriaceae strains that are resistant to any of the carbapenems in the infectious diseases and clinical microbiology laboratory of a 1500 bed university hospital. The aim of the study was to identify the resistance mechanism(s) of carbapenems in these strains and to check for clonal dissemination.
Methods: Bacterial strains and and MIC determinations. All the strains (15 *Klebsiella pneumoniae*, 1 *Escherichia coli*, 2 *Enterobacter* cloaceae and 2 *Enterobacter aerogenes*) were clinical isolates from the Istanbul University, Cerrahpasa Medical Faculty Hospital, Infectious Diseases and Clinical Microbiology Laboratory. The strains were identified with the API 32GN system. Antibiotic susceptibilities and MICs were with disc diffusion assay and E-test.
IEF analysis. Isoelectric focusing (IEF) analysis was performed with an ampholine polyacrylamide gel (pH 3.5 to 9.5).
PCR experiments and DNA sequencing: Using total DNA of each of the strains, PCR amplifications of the blaOXA-48 genes were performed with the primers OXA-48A (5′-TTGGTGGCATCGATTATCGG-3′) and OXA-48B (5′-GAGCACTTCTTTTGTGATGGC-3′), giving rise to a 743-bp fragment.
Molecular typing. Molecular characterisation of the strains was done by macrorestriction analysis of genomic DNA with XbaI (New England BioLabs). DNA fragments were separated by pulsed-field gel electrophoresis (PFGE) in a CHEF-DR II system (Bio-Rad). Electrophoresis conditions were pulse times ranging from 5 to 35 s for 24 h at 6 V/cm and 14°C.
Infection control procedures. Patients from whom carbapenem-resistant Enterobacteriaceae was recovered at any site were visited by a member of the infection control team. Colonisation or infection was determined according to the definition of the Centers for Diseases Control and Prevention for nosocomial infections.
Results and Conclusion: All the carbapenem resistant strains isolated produced OXA-48, a class D oxacillinase significant with carbapenem-hydrolysing activity. No relationship was observed among the seven patients hospitalised in different wards.

P1518 Diversity of antibiotic resistance determinants among the recent population of *Acinetobacter baumannii* strains belonging to European clone II

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Objective: The recent increase in *Acinetobacter baumannii* resistance to carbapenems in the Czech Republic has been associated with the spread of strains belonging to European clone II. Although multidrug resistance was a common property of these strains, they differed in resistance to particular agents. The aim of this study was to assess the genetic basis of this variation.
Methods: Sixty-six strains obtained from 37 intensive care units in the Czech Republic in 2005–6 were studied. The strains were previously classified as European clone II by AFLP and were shown to have highly similar or identical PFGE patterns. They were tested for MICs to sulbactam, ceftazidime, meropenem, gentamicin, tobramycin, amikacin, netilmicin and doxycycline, and for the presence of 20 genes associated with resistance to these agents.
Results: All strains were positive for the genes encoding OXA-51, AmpC and the AdeABC efflux system while no strain tested positive for those encoding metallo-β-lactamases, OXA-23 and OXA-24 carbapenemases, or aminoglycoside-modifying enzymes AAC(3)-II, AAC(6′)-I, and ANT(2″)-I. The strains varied with respect to the presence of the genes encoding the following proteins (% positive strains): TEM-1 (80), Tet(B) (92), Tet(A) (5), AAC(3)-I (83), APH(3′)-I (80), APH(3′)-VI (30), OXA-58 (3) and a class 1 integrase (83). ISAba1 was found in 95% strains and three integron variable regions (2.5, 3.0 and 3.5 kb) were identified, differing only in the number of copies of the orfX cassette. The presence of particular genes and the corresponding resistant phenotype were in good agreement. All strains with ISAba1 located upstream of the AmpC gene (n = 33) were resistant to ceftazidime (MIC > 64 mg/l) while those with ISAba1 upstream of the OXA-51 gene (n = 33) showed increased meropenem MICs (2–32 mg/l). Individual strains carried from 4 to 12 resistance genes in 17 combinations. Different combinations were also found in isolates from the same ward and having identical PFGE patterns.
Conclusion: The variation in antibiotic resistance in the studied strains results from the differences in the presence of acquired resistance genes and, possibly from the effect of ISAbaI on the expression of intrinsic genes. The high genetic versatility of European clone II might contribute to its ability to develop resistance to nearly all clinically relevant antibiotics.
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Quinolone and fluoroquinolone resistance – Part 2

P1519 Plasmid-mediated quinolone resistance determinant QepA from ESBL-producing *Escherichia coli* in France

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Objectives: Three different plasmid-mediated quinolone resistance (PMQR) determinants have been described: Qnr, AAC(6′)-Ib-cr and QepA. QepA is an efflux pump responsible for decreased susceptibility to hydrophilic fluoroquinolones, was identified in association with RmtB (responsible for resistance to all aminoglycosides) in two *Escherichia coli* isolates from Belgium and Japan. To date, the single epidemiological survey performed reported a low prevalence of the qepA gene (0.3%) among *E. coli* isolates from Japan. The aim of our study was to determine the presence of qepA gene among ESBL-producing enterobacterial isolates from a French University hospital.
Methods: Screening was performed on 121 ESBL-producing enterobacterial isolates collected in France from January 2007 to October 2007. PCR with primers specific for the qepA gene was used for screening. Detection and identification of ESBL genes was performed using specific

primers of sequences encoding TEM-, SHV- and CTX-M-type ESBLs. The presence of qnr-like and aac(6′)-Ib-cr genes were also searched for qepA-positive isolate. Plasmid extraction was performed by the Kieser method and transfer of plasmids to *E. coli* refernce strain was performed by electroporation.

Results: Out of 121 ESBL-positive enterobacterial isolates tested, a single *Escherichia coli* isolate (0.8%) was found to possess the qepA gene. Sequence analysis revealed a perfect identity with the previously published qepA. The qepA-positive *E. coli* clinical isolate was recovered from urine and blood samples of a patient suffering from pyelonephritis. This isolate carried the ESBL gene blaCTX-M-15 and also the narrow-spectrum penicillinase blaTEM-1 gene. That isolate was fully susceptible to aminoglycosides but showed a high level of resistance to quinolones and fluoroquinolones. PCR showed that this isolate did not possess Qnr-type and AAC(6′)-Ib-cr determinants. Transfer of the qepA-positive plasmid was obtained in an *E. coli* recipient isolate, giving rise to reduced susceptibility to norfloxacin and ciprofloxacin, and co-resistance to penicillins, chloramphenicol, tetracycline, and sulfonamides, but did not express an ESBL phenotype.

Conclusion: This study identified plasmid-mediated quinolone resistance QepA determinant in France. By contrast to previous findings, the QepA determinant was not associated with plasmid-mediated aminoglycoside resistance RmtB determinant.

P1520 Plasmid-mediated quinolone resistance genes in extended-spectrum β-lactamase-producing Enterobacteriaceae, Italy

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Objectives: At least 3 types of plasmid-mediated quinolone resistance (PMQR) mechanisms have emerged in Enterobacteriaceae, including Qnr proteins (which protect the topoisomerase target), the AAC(6′)-Ib-cr enzyme (which inactivates some quinolones by acetylation), and the QepA pump (which can efflux hydrophilic quinolones). The role of these mechanisms in evolution of fluoroquinolone resistance has been recognised, but current knowledge on their epidemiology is still limited. Here we studied the prevalence and distribution of PMQR determinants among Extended-Spectrum β-lactamase- (ESBL-) producing Enterobacteriaceae from an Italian nationwide survey.

Methods: 271 consecutive nonreplicate ESBL-producing-isolates of Enterobacteriaceae (*Escherichia coli*, *Klebsiella* spp. and *Enterobacter* spp.) were obtained from 11 clinical microbiology laboratories located in different Italian regions during the second ESBL national survey. Isolates were analysed by colony-blot hybridisation using DNA probes containing the gene aac(6′)-Ib or qepA or a mixture of qnrA, qnrB and qnrS genes. The hybridisation-positive isolates were further analysed by PCR-RFLP and sequencing.

Results: Overall, 62 (23%), 10 (4%) and none of the ESBL producers were found to harbour the aac(6′)-Ib-cr, qnr (either qnrA or qnrB) and qepA genes, respectively. aac(6′)-Ib-cr and qnr genes were widespread across the Italian territory (detected in 6 and 4 of the 11 centres, respectively). The former was mostly detected in *E. coli* (61 isolates, 42% of ESBL-producing *E. coli*) and occasionally in *Klebsiella pneumoniae* (one isolate, 1% of the ESBL-producing *K. pneumoniae*). In most cases aac(6′)-Ib-cr was associated with CTX-M-15 (81%), and in a minority of cases with a TEM-type ESBL (16%) or with SHV-12 (3%). qnrB gene was detected in *K. pneumoniae* (4 isolates, 6% of ESBL-producing *K. pneumoniae*), and qnrA in *Enterobacter cloacae* (6 isolates, 46% of ESBL-producing *E. cloacae*). Qnr genes were mostly associated with SHV-12 (80%), and in a minority of cases with a TEM-type ESBL (20%). No association between aac(6′)-Ib-cr and qnr was found.

Conclusions: Of PMQR genes, only aac(6′)-Ib-cr, qnrA and qnrB were detected among ESBL-producing isolates of Enterobacteriaceae circulating in Italy.

P1521 Prevalence of aac(6′)-Ib-cr gene in *Escherichia coli* isolated in northern Italy from patients with urinary tract infections

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Objectives: The allelic variant aac(6′)-Ib-cr of aac(6′)-Ib gene has been associated with resistance not only to aminoglycosides but also to fluoroquinolones such as norfloxacin and ciprofloxacin. Furthermore, the presence of the aac(6′)-Ib-cr gene has been associated with that of other resistance genes, such as blaCTX-M, encoded by the same plasmid. To estimate the prevalence of this variant and its spread in our geographical area, a retrospective study was conducted on a collection of urinary strains of *E. coli* isolated in northern Italy in 2004 and 2006.

Methods: 236 and 240 *E. coli* strains, isolated from urine cultures in the period November-December 2004 and in the same period of 2006, respectively, in San Gerardo Hospital of Monza (MI), were screened.

The presence of the aac(6′)-Ib-cr gene was evaluated by PCR followed by amplicons restriction using BstF5I. Unrestricted PCR products were sequenced to confirm the presence of the allelic variant. A multiplex PCR, to determine the presence of the qnr genes, was also performed. The presence of blaCTX-M has also been evaluated.

Results: Positivity for aac(6′)-Ib-cr gene was shown overall in 3 and 8 *E. coli* strains isolated in 2004 and 2006, respectively.

All strains shown to possess the aac(6′)-Ib-cr gene were found to have MIC values to norfloxacin and ciprofloxacin of >16 and >4 mg/L, respectively. None of the qnr genes were detected in aac(6′)-Ib-cr positive strains. Moreover, all aac(6′)-Ib-cr positive strains were also shown to possess blaCTX-M group 1 genes.

Conclusion: The prevalence of the aac(6′)-Ib-cr gene in isolates of *E. coli* characterised by norfloxacin MIC values ⩽1 mg/L, raised from 6.1% (3/49) in 2004 to 12.5% (8/64) in 2006, indicating a possible spread of strains characterised by the presence of this gene in northern Italy. The presence of the aac(6′)-Ib-cr gene was shown to be associated with resistance to fluoroquinolones in the isolates analysed. Moreover, a correlation between the presence of aac(6′)-Ib-cr and the blaCTX-M group 1 genes was also observed.

This represents the first study that demonstrates the presence and diffusion of this resistance determinant in our geographic area, although further epidemiological and molecular surveys are necessary.

P1522 Prevalence of qnr genes in Italy among *Salmonella* strains with reduced susceptibility to ciprofloxacin

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Objectives: Resistance to quinolones commonly develops by spontaneous point mutations on chromosomal genes. Recently, plasmid-mediated resistance of Qnr type has been identified in several enterobacterial species. This work aims to evaluate the presence of qnr genes among human strains of *Salmonella* spp. collected in Italy.

Methods: Ciprofloxacin MICs, was determined for 160 strains of *S. enterica* isolated in Italy during 2004–2006 and chosen randomly among 1811 human isolates already tested for antibiotic susceptibility, by disc diffusion method. To identify mutations responsible for ciprofloxacin resistance, the quinolone resistance-determining regions (QRDR) of gyrA, gyrB, parC, parE, were amplified by PCR and sequenced. Strains resistant or with reduced susceptibility to ciprofloxacin (MIC⩽0.125 μg/ml), were tested by PCR for the presence of qnrA, qnrB and qnrS genes. Strains positive for qnr genes were tested for the ability to transfer resistances by conjugation; plasmid DNA was subsequently used in cloning experiments to investigate the genetic environment of qnr gene.

Results: Fifty-eight of the 160 strains showed reduced susceptibility to ciprofloxacin, 3 strains (1 S. Typhimurium and 2 S. Kentucky) were resistant showing the same mutations in the QRDRs, as previously described.

One S. Typhimurium strain, resistant to ampicillin, cefotaxime, ceftazidime, kanamycin, trimethoprim/sulfamethoxazole, with a Cip

MIC=0.38 µg/ml, carried a qnrB gene showing 99% of aminoacidic identity with the sequence of qnrB10 and 98% of identity with qnrB5 found in S. Berta. No mutation in the QRDRs was found. Conjugation experiments revealed that all resistances were successfully transferred, except trimethoprim/sulfamethoxazole. Detailed plasmid characterisation of 16 kb region conferring multidrug resistance, identified the qnrB gene flanked by ISEcp1 and IS26, a blaSHV-12 gene flanked by two open reading frame of unknown function and by IS26, a blaTEM1 gene followed by truncated class1 integron, containing aadB gene cassette.
Conclusion: The plasmid-mediated quinolone resistance of the Qnr type is emerging in Enterobacteriaceae in many different geographic areas, but it remains rare in *Salmonella* in Italy. However the presence of qnrB genes in S. Typhimurium, never been described before, deserves particular attention because of the high prevalence of human infections due to this ubiquitous serotype.

P1523 Prevalence of the quinolone-modifying enzyme aac(6′)-Ib-cr in extended-spectrum β-lactamase-producing enterobacterial isolates in Barcelona

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Background: Quinolone resistance usually results from mutations in chromosomal genes coding for type II topoisomerases, efflux pumps, and/or porins alterations. Recently, plasmid-mediated quinolone resistance mechanisms has been reported by the qnr and the aac(6′)-Ib-cr genes.
Objective: To evaluate the presence of aac(6′)-Ib-cr among enterobacterial isolates carrying ESBLs in Barcelona, determine the ESBL present in each isolate and establish if the aac(6′)-Ib-cr and the ESBL genes were located in the same plasmid.
Methods: A total of 305 non-duplicate clinically relevant ESBL-producing enterobacterial isolates, obtained at Hospital Vall d'Hebron (Barcelona), collected between 2003 and 2004 were analysed for the presence of aac(6′)-Ib. Antimicrobial susceptibility was tested by Etest and disc diffusion. The screening for the presence of aac(6′)-Ib was studied by PCR using specific primers. Positive amplicons were digested with BstCI and sequenced to identify the aac(6′)-Ib-cr variant. ESBLs of all aac(6′)-Ib-cr-positive isolates were determined by IEF and PCR using specific primers. The quinolonone-resistance-determining region (QRDR) of gyrA and parC genes was amplified and sequenced. Plasmid number and location of aac(6′)-Ib-cr and bla genes was performed on all aac(6′)-Ib-cr-positive isolates by S1 nuclease digestion and hybridisation with specific probes.
Results: Nineteen isolates (6.2%) carried aac(6′)-Ib (8 *Escherichia coli*, 7 *Klebsiella pneumoniae*, 3 *Enterobacter cloacae* and 1 *Klebsiella oxytoca*). Of these, 6 isolates (31.6%) carried the aac(6′)-Ib-cr variant (5 *E. coli* and 1 *K. pneumoniae*). Among the 6 aac(6′)-Ib-cr-positive isolates 4 were positive for CTX-M-1 group. The ESBLs of the remaining two isolates are currently been determined. The 5 aac(6′)-Ib-cr-positive *E. coli* isolates were resistant to nalidixic acid and ciprofloxacin and the *K. pneumoniae* aac(6′)-Ib-cr-positive isolate was susceptible. Sequencing of the QRDR of the gyrA and parC genes identified amino acid changes in gyrA (S83-L and D87-N) and parC (S80-I and E84-V) on the 5 *E. coli* isolates. In the *K. pneumoniae* isolate no aminoacid changes were detected. The 4 aac(6′)-Ib-cr-positive isolates positive for CTX-M-1 group harboured the ESBL gene and aac(6′)-Ib-cr located in the same plasmid.
Conclusion: The prevalence of aac(6′)-Ib-cr, responsible for low-level quinolone resistance, among enterobacterial clinical isolates carrying ESBLs between 2003 and 2004 in Barcelona was 1.9%.

P1524 Emergence and dissemination of plasmid-borne fluoroquinolone resistance genes and their association with blaCTX-M genes in Enterobacteriaceae isolates from Portugal

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Objectives: Considering the increasing resistance to fluoroquinolones among the Enterobacteriaceae and the alarming mobility of some of the genes responsible, we aimed to understand the level of association between different plasmid-mediated fluoroquinolone resistance (PMFR) genes in Portuguese isolates and the correlation between these genes and a number of β-lactamase genes.
Methods: We screened two collections of Enterobacteriaceae isolates for the presence of qnrA, qnrB, qnrS and aac(6′)-Ib-cr. The first collection consisted of 104 isolates gathered in 1999 from 16 different Portuguese hospitals (namely 57 *Klebsiella pneumoniae*, 42 *Escherichia coli*, 4 Kluyvera spp. and 1 *Hafnia alvei*) and the second collection consisted of 467 isolates gathered from 15 hospitals in the period 2004–2006 (namely 314 *E. coli*, 88 *K. pneumoniae*, 41 *Enterobacter* spp., 6 *Klebsiella oxytoca*, 6 *Serratia marcescens*, 6 *Morganella morganii*, 3 *Citrobacter freundii*, 2 *Proteus mirabilis* and 1 *Kluyvera* spp.). All isolates from 2004–2006 were identified as extended-spectrum β-lactamase (ESBL) producers. PCR and nucleotide sequencing were used with specific primers for the PMFR genes and for the β-lactamase genes (blaTEM, blaSHV, blaOXA, blaCTX-M and ampC).
Results: We observed an emergence of qnrB (from 0% to 10%), qnrS (from 0% to 8%) and aac(6′)-Ib-cr (from 14% to 65%) from 1999 to 2004–2006, the latter's more evident in *E. coli* (from 19% to 79%); qnrB was mainly found in *K. pneumoniae* isolates (36%). We also observed that, while the other genes seem to be evenly distributed across the country, the frequency of qnrS is 17% in the North region, 5% in the Centre region and 1% in the Lisbon and Tagus Valley region. The frequency of blaCTX-M and blaOXA in isolates positive for aac(6′)-Ib-cr was 80% and 76%, respectively; in isolates negative for aac(6′)-Ib-cr this frequency was only 32% and 17%, respectively. The gene blaSHV was more frequent in positive isolates for qnrB (69%) than in negative (14%). There was also a higher frequency of qnrB in qnrS positive isolates (22%) than in qnrS negative ones (10%).
Conclusion: The emergence of PMFR genes over time contradicts previous reports and the uneven distribution of qnrS further implies a dissemination process. There seems to be a close association between aac(6′)-Ib-cr and the ESBL gene blaCTX-M. These findings reinforce a more careful use of fluoroquinolones and an increased monitorisation of their related resistance mechanisms.

P1525 Aquatic environment contamination with an epidemic *Escherichia coli* clone harbouring blaCTX-M-15, blaOXA-1, blaTEM-1, and aac(6′)-Ib-cr in Portugal

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Objectives: The presence of CTX-M-15-producing isolates in non-clinical compartments has been scarcely investigated. In this study we describe the presence of
CTX-M-15-producing *Escherichia coli* isolates in aquatic environments in Portugal. We also compared its clonal relationship with CTX-M-15-producing isolates recovered from clinical settings in the same geographic area.
Methods: We studied two CTX-M-15-producing *E. coli* isolates recovered from sea and streams reaching the shore, in Porto area, Portugal, during 2006. Water samples were plated on MacConkey agar with and without 1 mg/L ceftazidime or cefotaxime after filtration. Species identification, susceptibility testing and conjugation assays were performed by standard methods. ESBL characterisation included synergy test, IEF, and identification of known bla genes by PCR and sequencing. Clonal relatedness was established by RAPD-PCR. The RAPD patterns

were also compared with those obtained in CTX-M-15-producing *E. coli* clinical strains. Multilocus sequence typing (MLST) using the standard seven housekeeping loci was also performed. *E. coli* phylogenetic groups were identified by multiplex PCR. Presence of specific resistance genes was searched by PCR [blaOXA, blaTEM, blaCTX-M, aac(6′)-Ib].

Results: Both CTX-M-15-producing *E. coli* belonged to phylogenetic group B2 and were clonally related by RAPD analysis. Moreover, they shared the same RAPD pattern of the predominant Portuguese *E. coli* clinical clone, producing CTX-M-15 and belonging to clonal complex ST131. Both strains were resistant to β-lactams (except carbapenems), tetracycline, kanamycin, tobramycin and ciprofloxacin. blaTEM-1 and aac(6′)-Ib-cr-blaOXA-1 were also detected. Transfer of CTX-M-15 was achieved in both isolates.

Conclusions: Aquatic contamination with an epidemic and multiresistant B2 CTX-M-15-producing *E. coli* clone belonging to clonal complex ST131 is a matter of concern. This fact will undoubtedly increase the possibilities of dissemination of this epidemic clone and the corresponding blaCTX-M-15 plasmid. Control of release of these bacteria to the environment should be a public health priority in our country.

P1526 Environmental emergence of multiresistant Enterobacteriaceae harbouring blaCTX-M-15, and aac(6′)-Ib-cr in Portugal

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Objectives: Since our previous reports of seawater contamination with ESBL producers, we noticed a change in resistance phenotype in the last years, with emergence of CTX-M profile, in 2004, and increased resistance to fluorquinolones, in 2006. Spreading of CTX-M-15 producers in the clinical setting, has already been reported in Portugal in association with TEM-1, OXA-1 and AAC-(6′)-Ib-cr. In that way, it was our purpose the detection of CTX-M-15 and AAC-(6′)-Ib-cr producing isolates, in pluvial water streams reaching the shore and coastal seawater.

Methods: Pluvial water streams reaching the sea and seawater were collected from 2004 to 2006, from 3 beaches of the Porto area, in Portugal. Isolates were selected by membrane filtration technique and the filters were placed on MacConkey agar with and without ceftazidime (2 mg/l) or cefotaxime (2 mg/l). Lactose fermenters were randomly selected and susceptibility was determined according to the CLSI guidelines. Screening for ESBL producers was performed by the double disk synergy test. Identification was achieved by ID 32 GN. β-lactamases were characterised by isoelectric focusing and, in representative isolates, were identified by PCR and sequencing. Conjugation assays were performed with *Escherichia coli* HB101 and K802N.

Results: Forty six multiresistant Enterobacteriaceae isolates (mostly *E. coli* and *Klebsiella pneumoniae*), producing CTX-M-15 in different combinations with TEM-1, OXA-1 and AAC-(6′)-Ib-cr, were recovered from pluvial water streams presenting faecal contamination and seawater. Fifteen isolates were able to transfer the CTX-M-15 gene, by conjugation.

Conclusion: The presence of CTX-M-15 and AAC-(6′)-Ib-cr producers, in pluvial water streams and seawater, seems to reflect unexpected contamination by wastewater related to the healthcare setting, like hospitals, tertiary care institutions, long term care and nursing homes. This situation seems relevant in terms of public health and environmental protection. The incoming of this kind of multiresistant ESBL producers to natural environments and the transferability of the ESBL gene by conjugation, might provide a track for environmental dissemination of resistant bacteria and genes that may create a source of transferable traits for environmental bacteria, influencing natural reservoirs of resistance with possible transference for emerging pathogens.

P1527 Molecular epidemiology of plasmid-mediated quinolone resistance determinants in extended-spectrum β-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* isolates from Turkey

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Objectives: To evaluate the distribution of plasmid-mediated quinolone resistance (PMQR) of the Qnr and aminoglycoside acetyltransferase AAC(6′)-Ib-cr types, among clinically-extended-spectrum β-lactamase (ESBL) producing enterobacterial isolates recovered from blood cultures of patients in different Turkish hospitals.

Methods: 248 ESBL-producing *Escherichia coli* and *Klebsiella* sp. isolates were collected from blood cultures from January to December 2006 in nine Turkish hospitals. ESBL-positive isolates were detected according to Clinical and Laboratory Standards Institute (CLSI) criteria. The bacterial isolates were tested for qnrA, qnrB, qnrS and aac(6′)-Ib-cr genes by a PCR technique. β-Lactamase-encoding genes were detected by PCR using primers for detection of TEM, SHV, OXA and CTX-M variants. Conjugation experiments were performed to determine whether the plasmids carrying the qnr or aac(6′)-Ib-cr genes were self-transferable. Genetic structures surrounding the qnr gene were analysed by PCR and clonings.

Results: A total of 138 and 110 ESBL-producing isolates were identified as being *E. coli* and *K. pneumoniae* sp., respectively. Sixty-three percent of the 248 ESBL-producing isolates were resistant to nalidixic acid. Multiplex PCR-screening detected a single *K. pneumoniae* isolate harbouring a qnrB1 gene (0.4%), whereas no qnrA or qnrS gene was detected. The qnrB1-positive isolate was also positive for the blaCTX-M-15, blaSHV-12 and aac(6′)-Ib-cr genes. The qnrB1-positive plasmid was 124-kb in size, co-harboured the blaSHV-12 gene, and attempts to transfer by conjugation failed. The blaCTX-M-15-positive plasmid co-harboured the aac(6′)-Ib-cr gene. Out of 50 ESBL-producing isolates tested, 39 (78%) were positive for the aac(6′)-Ib-cr variant. All isolates carrying the aac(6′)-Ib-cr gene were resistant to ciprofloxacin and two of them, two *K. pneumoniae* isolates, were resistant to imipenem due to the production of β-lactamase OXA-48.

Conclusion: This study constitutes an epidemiological survey of PMQR determinants among ESBL enterobacterial isolates from highly significant clinical samples (blood isolates) and shows a high prevalence of AAC(6′)-Ib-cr determinants in Turkey. However Qnr determinants are very rarely identified here.

P1528 Low prevalence of plasmid-mediated quinolone resistance in Norwegian and Swedish clinical isolates of *Escherichia coli* and *Klebsiella* spp.

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Objectives: The objective of this study was to examine the prevalence of the plasmid-mediated resistance genes, qnr and aac(6′)-Ib-cr, among Norwegian and Swedish clinical isolates of *Escherichia coli* and *Klebsiella* spp. with reduced susceptibility to ciprofloxacin and/or resistance to nalidixic acid.

Methods: 487 isolates of *E. coli* (n = 326) and *Klebsiella* spp. (n = 31) from (i) Kronoberg Sweden (n = 352) isolated between 2004–5, (ii) the Norwegian surveillance programme for antimicrobial resistance (NORM) (n = 318) isolated in 2005 and (iii) ESBL- (n = 304) or AmpC-producing (n = 33) isolates from the Norwegian Reference Centre for Detection of Antimicrobial Resistance (K-res) collected between 2003–5, were screened for the presence of qnr and aac(6′)-Ib-cr genes by multiplex and single PCRs, respectively. PCR products were confirmed by sequencing and the aac(6′)-Ib-cr variant was also identified by BstCI-digestion. The genetic environment of qnrS positive isolates was examined by targeted PCR and sequence analysis. Transfer of qnr to *E. coli* J53 RifR was examined.

Results: Qnr-like encoding genes were identified in 8/487 isolates (1.6%), five *Klebsiella* spp. (8.2%) and three *E. coli* (0.7%). Six isolates were qnrS1 positive; Kronoberg (n = 3), NORM (n = 3), and K-res (n = 3) collections. Two isolates were qnrB positive (qnrB1 and qnrB7-like) in the K-res collection. Interestingly, four qnr positive isolates also harbored the aac(6′)-Ib-cr variant. Transfer of the qnrS (n = 3) or qnrB (n = 3) genes to *E. coli* J53 was obtained using 100 mg/L ampicillin as selection pressure indicating plasmid association and co-transfer with a β-lactamase gene. The genetic surroundings of the qnrS1 gene were similar in the three isolates examined. ISEcl2 (orfB ÄorfA) were detected upstream and Äres, orf259, and orf213 were detected downstream of the qnrS1 gene. However, one of the isolates had a further truncated Äres gene. One isolate also carried the pbp3 and blaLAP-1 genes upstream of the qnrS1 gene. The aac(6′)-Ib gene was detected in 76/487 isolates (15.6%). 66 (86.8%) of these were of the aac(6′)-Ib-cr variant.
Conclusions: (i) The prevalence of qnr (1.6%) and aac(6′)-Ib-cr (13.6%) genes are low in Norwegian and Swedish clinical isolates of *E. coli* and *Klebsiella* spp. with reduced susceptibility to fluoroquinolones, as already observed in other European countries. (ii) The genetic context of the qnrS1 gene in three isolates showed similar structures as previously described.

P1529 Prevalence and genetic environment of aac(6′)-Ib-cr in AmpC-producing *Enterobacter cloacae*, *Citrobacter freundii* and *Serratia marcescens*: a multicentre study from Korea

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Objectives: To investigate the prevalence of aac(6′)-Ib-cr, their association with other plasmid-mediated resistance genes, and their influence on quinolone susceptibility in AmpC-producing Enterobacteriaceae without any selection criteria.
Methods: A total of 479 consecutive, non-duplicate isolates of *Enterobacter cloacae* (179), *Citrobacter freundii* (134) and *Serratia marcescens* (166) collected from 12 clinical laboratories without any selection criteria, which we previously studied for the presence of qnr determinants, were examined. We performed PCR for aac(6′)-Ib gene, blaoxa-1, ISEcp1, IS26. The data for qnr genes, ESBL (blaTEM, blaSHV, blaCTX-M), and orf513 were applied from the previous study. To determine the cr variant of aac(6′)-Ib, all the positive isolates were further analysed with BstCI digestion. A Mantel-Haenszel Chi2 test was used for the comparison of dichotomous variables.
Results: The aac(6′)-Ib was detected in 110 (23%) of 479 Enterobacteriaceae; 74 (41.3%) of 179 *E. cloacae*, 25 (18.7%) of 134 *C. freundii*, 11 (6.6%) of 166 *S. marcescens*. Among these 110 aac(6′)-Ib-positive isolates, cr variant was detected in 15 (13.6%) of isolates; 8/74 (10.8%) of *E. cloacae*, 5/25 (20%) of *C. freundii*, 2/11 (18.2%) of *S. marcescens*. Blaoxa-1 and orf513 were significantly associated with aac(6′)-Ib-cr in all three species, whereas the association with blaCTX-M, qnr determinants, ISEcp1, and the resistance to quinolones was variable among species. Eleven of 15 aac(6′)-Ib-cr producers harboured qnr genes (6 qnrA, 4 qnrB, 1 qnrS).

Table 1. Characteristics of aac(6′)-lb-cr-positive and aac(6′)-lb-cr-negative isolates

Characteristic[a]	*E. cloacae*			*C. freundii*			*S. marcescens*		
	cr(+) [n = 8] n (%)	cr(–) [n = 171] n (%)	P	cr(+) [n = 5] n (%)	cr(–) [n = 129] n (%)	P	cr(+) [n = 2] n (%)	cr(–) [n = 164] n (%)	P
ESBL	3/8 (37.5)	56/171 (32.7)	0.78	2/5 (40)	2/129 (1.6)	**0.00**	0/2 (0)	21/164 (12.8)	0.59
CTX-M	3/8 (37.5)	35/171 (20.5)	0.25	2/5 (40)	1/129 (0.8)	**0.00**	0/2 (0)	13/164 (7.9)	0.68
SHV ESBL	2/8 (25)	34/171 (19.9)	0.72	0/5 (0)	1/129 (0.8)	0.84	0/2 (0)	9/164 (5.5)	0.73
Plasmid TEM	0/7 (0)	31/171 (18.1)	0.22	1/5 (20)	12/129 (9.3)	0.43	0/2 (0)	25/164 (15.2)	0.55
OXA-1	7/8 (87.5)	9/171 (5.3)	**0.00**	2/5 (40)	1/129 (0.8)	**0.00**	1/2 (50)	2/164 (1.2)	**0.00**
IS*Ecp1*	4/8 (50)	14/171 (8.2)	**0.00**	2/5 (40)	4/129 (3.1)	**0.00**	0/2 (0)	10/164 (6.1)	0.72
orf513	6/8 (75)	66/171 (38.6)	**0.04**	2/5 (40)	9/129 (7)	**0.01**	1/2 (50)	11/164 (6.7)	**0.02**
IS*26*	5/8 (62.5)	81/171 (47.4)	0.40	3/5 (60)	46/129 (35.7)	0.27	0/2 (0)	1/164 (0.6)	0.91
qnrA	5/8 (62.5)	21/171 (12.3)	**0.00**	0/5 (0)	1/129 (0.8)	0.84	1/2 (50)	0/164 (0)	**0.00**
qnrB	0/8 (0)	22/171 (12.9)	0.28	3/5 (60)	49/129 (38)	0.32	1/2 (50)	2/164 (1.2)	**0.00**
qnrS	1/8 (12.5)	1/171 (0.6)	**0.00**	0/5 (0)	0/129 (0)		0/2 (0)	0/164 (0)	
CIP-R	6/8 (75)	29/171 (17)	**0.00**	3/5 (60)	23/129 (17.8)	**0.02**	1/2 (50)	21/164 (12.8)	0.12
NA-R	8/8 (100)	65/171 (38)	**0.00**	5/5 (100)	42/129 (32.6)	**0.00**	1/2 (50)	83/164 (50.6)	1.00

[a] CIP, ciprofloxacin; NA, nalidixic acid; R, resistant.

Conclusions: The aac(6′)-Ib-cr was present in AmpC-producing Enterobacteriaceae in Korea and was associated with other resistance genes (blaoxa-1, blaCTX-M, and qnr determinants) and various mobile elements (orf513 and ISEcp1).

P1530 The prevalence and distribution of plasmid-mediated quinolone resistance genes in clinical isolates of *Escherichia coli*

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Objectives: Plasmid-mediated quinolone-resistance mechanisms in Enterobacteriaceae include the three genes of the qnr series (qnrA, B and S), and a variant aminoglycoside acetyltransferase (aac(6′)-Ib-cr) which increases resistance to tobramycin and amikacin, and inactivates ciprofloxacin and norfloxacin. Aac(6′)-1b-cr, initially described in Shanghai and the USA, is also found in CTX-M-15-bearing plasmids in *Escherichia coli* from the UK and Canada. Such CTX-M-15 producing strains of lineage ST-131 serotype O25 became prevalent in the UK in 2003. A rise in quinolone resistance in *E. coli* bacteraemias was reported in the UK in 2001. The aim of this study was to assess the presence of plasmid encoded quinolone resistance genes in *E. coli* outside CTX-M-15 producing strains both historically and currently.
Methods: Ciprofloxacin resistant *E. coli* non-ESBL-producing isolates included those collected nationally as part of the BSAC UK bacteraemia surveillance programme between 2001 and 2005, regional urine isolates collected in early 2000 and local urinary isolates collected in 2006. PCR was used to amplify and detect the generic aminoglycoside acetyltransferase gene (aac(6′)-Ib). The fluoroquinolone-modifying variant of the gene was identified using restriction fragment length polymorphism (RFLP). Multiplex PCR was used for detection of qnrA, B and S. Isolates with aac-(6′)-1b-cr had MIC tests carried out for amikacin and tobramycin and were serotyped.
Results: The prevalence of the aac(6′)-Ib-cr gene was 3% and 9% in respectively current urinary and historic bacteraemia quinolone-resistant non-ESBL isolates of *E. coli*. Of 521 urinary *E. coli* isolates from 2000, only 14 (2.9%) were norfloxacin resistant, none of which carried the aac(6′)-Ib-cr gene. Positive isolates from bacteraemia in 2001/2 were serotype O102 and in 2004/5, O1 and O25. Positive urinary isolates from 2006 included serotypes O1 and O25. MICs showed unusual amikacin susceptibility.
Conclusions: Aac(6′)-1b-cr now occurs in *E. coli* in the UK in the absence of CTX-M-15 but with a restricted serotype distribution. Its presence in bacteraemia isolates from 2001 and 2002, prior to the spread of CTX-M-15 in Britain might suggest either a common source species in which the gene occurred or a lineage from which plasmid-recombination occurred. As yet, there is no evidence that this mechanism of quinolone resistance was responsible for the rise in quinolone resistance in the UK from 1996 to 2001.

P1531 Prevalence of plasmid-mediated quinolone resistance determinants QnrA, QnrB, and QnrS among clinical isolates of *Klebsiella pneumoniae* in Scotland

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Introduction: Quinolone resistance results from mutations in the chromosomally-encoded type II topoisomerases, and via the upregulation of efflux pumps, or porin-related genes. Recent studies demonstrate that the plasmid-mediated qnr genes (QnrA, B and S) play an emerging role in the dissemination of fluoroquinolone resistance. The first plasmid-mediated quinolone resistance protein Qnr was identified in a *Klebsiella pneumoniae* isolate in 1994 where the presence of the qnr gene increased the MICs to nalidixic acid and fluoroquinolones by four- to eight-fold. The qnr gene has been identified in complex In4 family class 1 integrons, known as complex sul1-type integrons that may act as a recombinase for mobilisation of the antibiotic resistance genes located nearby (e.g., qnr, blaCTX-M and ampC). In addition, an association of quinolone

resistance with the production of ESBLs has been reported. This study aimed to determine the prevalence of qnr genes and its association with the presence of ESBLs in clinical *K. pneumoniae* isolates from Scotland.
Methods: Isolates of *K. pneumoniae* were collected from the Royal Infirmary Edinburgh in 2007. The qnrA, qnrB, and qnrS genes were detected by PCR. MICs of ciprofloxacin, nalidixic acid, cefotaxime, ceftazidime, ceftriaxone, cefoxitin and meropenem were performed according to the BSAC guidelines. ESBL production was confirmed by double and combination disk methods.
Results: Of the 95 isolates tested, 27 were found to be positive by PCR for the qnr genes. These positive isolates were further assessed by PCR where 11 possessed the qnrB gene, 10 contained the qnrA gene and 2 harboured the qnrS gene. Interestingly four isolates were found to harbour both the qnrB and qnrS genes. Out of the 27 qnr positive isolates, 12 (44.4%) isolates were found to be resistant to nalidixic acid, while the remaining 15 (55.6%) were sensitive to nalidixic acid. Six of the qnrB positive isolates were associated with CTX-M, SHV and TEM, one isolate with TEM and SHV β-lactamases. Two qnrA positive isolates were associated with TEM and SHV only. However of the isolates that were tested none were found to harbour an ESBL and the qnrS gene.
Conclusion: These findings indicate the high prevalence of qnr genes and the co-expression of fluoroquinolone and extended-spectrum β-lactam resistance among *Klebsiella pneumoniae* isolates in Scotland.

P1532 First report of plasmid-mediated quinolone resistance determinants qnrA, qnrB and qnrS among Gram-negative clinical isolates from Aveiro, Portugal

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Objectives: The aim of the present study was to evaluate the prevalence of qnr genes in a growing number of Gram negative quinolone resistant strains, in Hospital Infante D. Pedro, Aveiro. Relevance was given to *Escherichia coli* isolates causing urinary tract infections, given its importance in nosocomial and community acquired infections.
Methods: From January 2007 to October 2007, 80 quinolone resistant Enterobacteriaceae isolates were recovered from different inpatients of the Hospital Infante D. Pedro de Aveiro, Portugal. The isolates were identified by the automatic VITEK 2 system and Advanced Expert System (VITEK 2 AES) (BioMérieux, Marcy L'Étoile, France). Antibiotic susceptibilities were also determined by VITEK 2 and the susceptibility reports were edited after interpretation by AES of the inferred resistance phenotype. Presence of ESBL was confirmed by Etest® (AB Biodisk) ESBL with Cefotaxime/Cefotaxime + Clavulanic acid and Ceftazidime/Ceftazidime + Clavulanic acid strips, according to manufacturer's instructions. PCR, sequencing and sequence analysis was used to assess qnr encoding sequences. Sequences obtained were compared with others deposited in the GeneBank.
Results: qnrA and qnrB genes were detect in both *Escherichia coli* and *Klebsiella pneumoniae* isolates. However, qnrS was detected only in one *Escherichia coli* isolate, HIP15, that carries a class 1 integron, with genes conferring resistance to aminoglycosides and trimetoprim, positive for the presence of ISCR1 and for the extended-spectrum β-lactamase, TEM.
Conclusion: This is the first report of qnr genes in Portugal. As these genes are plasmid mediated their high incidence, particularly among *E. coli* strains, is disconcerting. Despite the fact that qnr genes being responsible for low-level of quinolone resistance, in presence of other chromosomally encoded resistance genes/mechanisms, they could be an important tool for bacteria to avoid lethal quinolone concentrations.
These results show the tremendous variety of different genetic determinants that can be found in one single isolate, thus contributing for the increasing levels of resistance. The genetic environment of the different resistance structures found is under study.

P1533 qnr genes in ESBL-producing Enterobacteriaceae isolates from New South Wales, Australia

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Objectives: To investigate the prevalence and diversity of qnr quinolone resistance genes in ESBL-producing isolates of Enterobacteriaceae in NSW.
Methods: A total of 78 ESBL-producing isolates were investigated, including 57 *Escherichia coli*, 15 *Klebsiella pneumoniae* and 6 belonging to other enterobacterial species all known to carry blaCTX-M, blaSHV-12 or blaVEB. qnrA, qnrB and qnrS were screened for by PCR. Mating experiments were performed for qnr-positive isolates. Clonal relatedness was determined by PFGE. A qnrB9 variant was cloned into the low copy vector, pBBR1-MCS1, to construct plasmid pBBR:qnrB9. MICs of norfloxacin, ciprofloxacin, gatifloxacin and moxifloxacin against the transformant with the qnrB9 gene were determined by E-test.
Results: qnrA1 was identified in 2 SHV-12-producing *K. pneumoniae* isolates with different PFGE patterns. qnrB1 was seen in one *K. pneumoniae* isolate carrying blaCTX-M-15. A qnrB1 variant with a deletion of A at position 19 was found in three *K. pneumoniae* also carrying blaCTX-M-15. Three isolates (1 *K. pneumoniae*, 1 *Klebsiella oxytoca* and 1 *Enterobacter* gergoviae) with blaCTX-M-62 also carried qnrB2. A single *Citrobacter freundii* isolate carrying blaCTX-M-15 had a qnrB9 variant with 5 nt silent differences compared with the qnrB9 gene in GenBank accession no. AM774477. Four *K. pneumoniae* isolates belonging to a single strain had qnrS1. Conjugative transfer of qnr genes was successful in 1/2 qnrA1-, 6/8 qnrB- and all 4 qnrS1-containing isolates. The MICs of norfloxacin, ciprofloxacin, gatifloxacin and moxifloxacin against a DH5 carrying pBBR:qnrB9 were 0.75, 0.25, 0.38 and 0.50 μg/ml, respectively. These were elevated 4–16 fold comparing with DH5 carrying pBBR1-MCS1 alone, indicating that QnrB9 conferred low-level resistance to quinolones.
Conclusions: qnrA, qnrB and qnrS genes have all been detected in ESBL-producing enterobacterial isolates in Australia, mainly associated with species other than *E. coli*. Several qnrB variants were found. The majority of these qnr genes were associated with conjugative plasmids which may explain their spread. As expected QnrB9 confers low-level resistance to quinolones.

P1534 First description of a plasmid-mediated quinolone resistance determinant qnrS2 in an *Aeromonas veronii* biovar *sobria* clinical isolate

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Objective: The main objective of this study was to investigate the prevalence of the qnrA, qnrB and qnrS genes in a collection of environmental and clinical *Aeromonas* sp.
Methods: Fifty seven *Aeromonas* sp. isolates (resistant and susceptible to nalidixic acid) were identified by biochemical tests. Thirty two were collected from the environment and the other twenty five were clinical isolates. Screening of the qnrA, qnrB and qnrS genes was performed by multiplex PCR using specific primers and controls for each gene. The sequence obtained was compared with those published in the GenBank. Antimicrobial susceptibilities were performed by E-test and gyrA analyses was carried out by PCR of the QRDR in the qnr+-PCR isolates. Plasmid extraction was carried out in the qnr-carrying strain.
Results: Among all the analysed isolates only one of them was PCR+. The positive strain corresponded to an *Aeromonas veronii* biovar *sobria* clinical isolate. It presented two mutations, one in the gyrA gene (Ser83-Ile) and the other in the parC gene (Ser80-Ile). The qnr determinant was sequenced and presented 100% homology when compared with the qnrS2 gene in the GeneBank. The positive isolate had a clinical origin and it showed resistance to nalidixic acid (>256 μg/ml), ciprofloxacin (8 μg/ml) and norfloxacin (12 μg/ml). A 8 Kb plasmid was isolated from the strain carrying the qnr-like determinant. The plasmid was cut off

from the agarose gel and the PCR of the qnrS2 gene repeated, showing the same amplification fragment.
Conclusions: This is the first time that a plasmid-mediated quinolone resistance qnr-like determinant has been found outside Enterobacteriaceae and in an *Aeromonas veronii* biovar *sobria* clinical isolate.

P1535 **Prevalence of the plasmid-mediated quinolone resistance determinant qnrS among ciprofloxacin-resistant *Escherichia coli* genotypes in Greece**

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Objectives: To determine the prevalence of the plasmid-mediated quinolone resistance qnr genes in resistant ciprofloxacin among *Escherichia coli* clinical isolates.
Methods: During the period 2005 to 2007, 114 ciprofloxacin-resistant *Escherichia coli* clinical isolates were collected from separate patients (the majority originated from urine cultures) hospitalised in four distant Greek hospitals. Isolates were screened for the presence of genes qnrA, qnrB and qnrS by PCR, using universal primers for each gene amplifying all related alleles. The qnrS-positive isolates were also screened by PCR for the detection of mutations in genes gyrA and parC. DNA-relatedness of the isolates of the study was examined by PFGE. Transferability potency of gene qnrS was investigated by conjugation.
Results: A total 12 out of the 114 tested isolates were positive for the qnrS1 gene but none for qnrA or qnrB gene. The 12 qnrS positive isolates were clonally unrelated and comprised at least 11 out of the 20 detected genotypes. Quinolone resistance was transferable with relatively low frequencies.
Conclusions: This is the first report of qnrS-positive *E. coli* in Greece. The proportion of approx. 10.5% of quinolone-resistant *E. coli* that carried qnr genes was considerably high in comparison with respective percentages from others regions, which ranged from 0.5 to 7.7%. All isolates belonged to unrelated clones, indicating that the spread of gene qnrS was not due to clonal dissemination.

P1536 **Mechanisms of resistance in non-typhoidal *Salmonella enterica* exhibiting a novel quinolone resistance phenotype**

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Objective: To determine the mechanisms which underpin a novel quinolone resistance phenotype of *Salmonella enterica* isolates from Finnish patients. All isolates were susceptible to nalidixic acid but had reduced susceptibility to ciprofloxacin.
Methods: Six *Salmonella enterica* isolates, consisting of five serovars (Corvallis, Stanley, Mbandaka, Montevideo and Virginia) were acquired by Finnish patients during travel to Malaysia and Thailand. Isolates were screened for plasmid mediated quinolone resistance by plasmid extraction and by PCR of qnrA, qnrB, qnrS, aac6′-lb-Cr, qepA and oqxB. Positive qnr genotypes were compared with previously identified qnr plasmids by Southern blotting. Transfer of resistance was examined by transformation and subsequent MIC testing of transformants. Mutations in the quinolone resistance determining regions (QRDRs) of gyrA, gyrB, parC and parE were identified by PCR and DHPLC. Efflux activity was assessed by determining accumulation of the fluorescent substrate Hoescht 33342 by all isolates and compared with matched wild strains of the same serovar.
Results: Plasmid mediated quinolone resistance genes were detected in all six isolates. One isolate carried qnrA, two qnrS and three both qnrB and qnrS. aac6′-lb-Cr, qepA and oqxB were not detected. Southern blotting identified qnrS1 in Stanley, Montevideo, Virginia and both Corvallis serovars. All plasmids, when transferred into a wild-type host had increased MICs of ciprofloxacin. In addition, plasmids from Stanley, Mbandaka and Montevideo serovars conferred increased MICs of chloramphenicol and tetracycline. No mutations were detected in the QRDRs of gyrA, gyrB, parC and parE, however, two isolates possessed mutations in gyrA at codon 396 (Leu to Met). All isolates accumulated less Hoescht 33342 than wild-type suggesting increased efflux activity.
Conclusion: These data demonstrate the rise of a highly mobile genotype that confers a novel quinolone resistance phenotype, which has become widely disseminated.

P1537 **Absence of plasmid-mediated quinolone resistance in *Salmonella* and *E. coli* strains from healthy cattle, pigs and chickens from 8 EU countries within the European Antimicrobial Susceptibility Surveillance in Animals (EASSA) programme**

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Objectives: In various countries, a variety of plasmid-encoded qnr genes conferring resistance to quinolones have been described in Enterobacteriaceae including *Salmonella* and *E. coli*. In previous EASSA studies, antimicrobial susceptibility to ciprofloxacin (CIP) has been investigated among *Salmonella* spp. and *E. coli* from food animals at slaughter across the EU. To assess the prevalence of qnr genes in the EASSA collection, all *Salmonella* and a selection of *E. coli* isolates with a decreased susceptibility to CIP were screened for qnr. Additionally, *E. coli* isolates showing differing levels of resistance were studied. As qnr genes are sometimes associated with strains that produce extended-spectrum β-lactamases (ESBLs), isolates with decreased susceptibility to cefotaxime (CT) were also included.
Methods: In all, 80 *Salmonella* and 77 *E. coli* exhibiting decreased susceptibility (MICs 0.12–2 mg/l) or resistance (MICs $\leqslant$4 mg/l) to CIP were selected from the EASSA collection 1999–2005. None of the *Salmonella* of the collection was clinically resistant to CIP. In addition, 13 *Salmonella* and 48 *E. coli* isolates with decreased susceptibility to CT (MICs 1–32 mg/l) or resistance (MICs $\leqslant$64 mg/l) were tested. Presence of qnr genes was determined by multiplex and simple PCRs for qnrA, qnrB and qnrS. A positive control for each qnr gene was included in each test. Strains were tested for susceptibility to nalidixic acid (NA) and phenotypically for ESBLs according to CLSI guidelines.
Results: None of the 93 *Salmonella* or the 125 *E. coli* isolates exhibited either of the qnr genes. Positive controls for qnrA, qnrB and qnrS were identified in each test. For *Salmonella*, two categories of NA susceptibilities were found: MICs $\leqslant$128 mg/l for 69 isolates (corresponding to CIP MICs from 0.12 to 0.5 mg/l) and MICs of 8 or 16 mg/l (11 isolates). Out of 13 *Salmonella* isolates with decreased susceptibility to CT, albeit susceptible to CIP, only one strain (S. Rissen) appeared to be a ESBL producer. For *E. coli*, NA MICs were $\leqslant$256 mg/l for 75 isolates (corresponding to CIP MICs from 0.12 to 64 mg/l), and were 64 mg/l for 2 isolates. Twenty-two strains were identified as ESBL producers.
Conclusions: In *Salmonella* and *E. coli* strains from healthy animals of this representative EU collection any qnr genes could not be detected. Currently, the prevalence of the qnr gene seems to be very uncommon. On-going surveillance including screening for qnr genes is however important.

Antimicrobial clinical trials

P1538 **Prospective, randomised clinical trial to compare the efficacy of two 70% (v/v) isopropyl alcohol solutions containing either 0.5% (w/v) chlorhexidine gluconate or 2% (w/v) CHG for skin antisepsis during coronary artery bypass grafting**

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Objectives: Whilst there has been insufficient research examining the effects of pre-operative skin antiseptics on surgical site infection (SSI) rates, current guidelines recommend 2% chlorhexidine gluconate (CHG) in 70% isopropyl alcohol (IPA) prior to insertion of central venous

access devices in NHS hospitals. Since most UK healthcare institutions currently use 0.5% (v/v) CHG in 70% (v/v) IPA for skin antisepsis prior to surgical procedures we are conducting a randomised clinical trial to compare the efficacy of these two antiseptics.

Methods: 70% (v/v) (IPA) containing either 0.5% (w/v) or 2% (w/v) CHG was applied to the proposed skin incision site made to harvest lengths of saphenous vein in patients undergoing coronary artery bypass grafting at University Hospital Birmingham NHS Foundation Trust. Skin swabs were taken from the incision sites pre-preparation, post-preparation and post-wound closure to assess the immediate and persistent efficacy of each antiseptic agent. Each specimen was immediately transferred into a chlorhexidine-neutralising solution and the microorganisms enumerated following microbiological culture. The number of patients who developed SSI was also determined.

Results: Microbiological specimens were recovered from 30 patients. The overall baseline bacterial loads pre-preparation from both patient groups were similar (p = 0.7). There was a greater reduction in the total numbers of microorganisms present on the skin 2 minutes post-skin preparation from baseline with the solution containing 2% CHG compared to 0.5% CHG (99.9% vs 97.3% p = 0.07). There was no difference between the two groups with regard to microbial numbers on the skin sampled at subsequent time periods. Four of 15 patients in the 0.5% CHG group developed a superficial SSI post-discharge from hospital compared to 0 of the 10 patients in the 2% CHG group (p = 0.1).

Conclusions: The results of this interim analysis suggest that 2% (w/v) CHG in 70% (v/v) IPA may outperform 0.5% (w/v) CHG in 70% (v/v) IPA in terms of reducing the total numbers of microorganisms on the skin and also the number of SSI which subsequently arise. 2% (w/v) CHG in 70% (v/v) IPA may therefore offer additional protection from SSI. Further study participants will be recruited.

P1539 Assessment of tolerability of 300 and 600 mg doses of faropenem medoxomil

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Objective: To compare the tolerability and safety of the 300 mg and 600 mg BID doses of faropenem medoxomil (FM), an oral penem antibiotic in development for the treatment of community respiratory infections.

Methods: A phase three program of FM (formerly faropenem daloxate) was conducted by Bayer AG from 2000–2002. Replidyne considered the 600 mg dose to be more appropriate for further development. Before initiating phase 3 studies with 600 mg BID dose, Replidyne compared the 300 mg and 600 mg dose to assess the relative adverse event (AE) profile focusing on gastrointestinal AEs. Study 002 was conducted in healthy volunteers, randomised to 300 mg or 600 mg (in a 1:2 ratio) BID for 7 days. Study 001 was then conducted in patients with acute bacterial sinusitis who underwent a baseline antral tap. Subjects received either 300 mg BID for 7 days or 600 mg BID for 5 days in a 1:1 ratio.

	FM 300 mg 7d [N = 122] N (%)	FM 600 mg 5/7d [N = 175] N (%)
Drug-related AEs	28 (22.9)	52 (29.7)
Gastro-intestinal disorders	20 (16.4)	31 (17.7)
Diarrhoea/loose stools	11 (9.0)	17 (9.7)
Flatulence	0	5 (2.8)
Abdominal pain	2 (1.6)	2 (1.1)
Dry mouth	1 (0.8)	3 (1.7)
Dyspepsia	1 (0.8)	1 (0.6)
Constipation	1 (0.8)	1 (0.6)
Vomiting	2 (1.6)	0

Results: The AEs for each dosage were combined and are shown in table. Overall, drug-related gastrointestinal AEs were similar 16.4% and 17.7% as were the rates of diarrhoea and loose stools for the 300 mg (9.0%) and 600 mg (9.7%) respectively. The AEs other than gastrointestinal were too infrequent to draw conclusions.

Conclusion: There is no difference in the adverse event profile for the 600 mg BID dosed subjects compared to subjects receiving 300 mg BID.

P1540 Levofloxacin 500 mg once daily oral in the treatment of chronic bacterial prostatitis

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Objectives: Levofloxacin is a fluoroquinolone that is active against a wide range of Gram-negative and Gram-positive bacteria. Levofloxacin has demonstrated efficacy in the treatment of complicated urinary tract infections. The study was aimed at further confirming the efficacy and safety of levofloxacin in chronic bacterial prostatitis (CBP) in Europe.

Methods: Men with a history of CBP, clinical signs and symptoms and laboratory evidence of prostatitis were enrolled in a prospective, multinational (8 countries) open-label study to receive levofloxacin 500 mg OD per os for 28 days. They were followed for 6 months.

Results: A total of 117 patients were treated (age 45 years, duration of CBP 48 months, median values). Gram-negative bacteria were identified in 57/106 patients (mainly *E. coli*, n = 37) and Gram-positive bacteria in 60/106 cases (mainly *Enterococcus faecalis*, n = 38 and *Staphylococcus epidermidis*, n = 34) with verified baseline pathogen.

Of the men evaluable for clinical outcome (ITT population), the clinical success rate (cured and improved patients, with 95%-CIs) was 92% [84.8, 96.5], 77.4% [68.2, 84.9], 66.0% [56.2, 75.0], and 61.9% [51.9, 71.2], at 5–12 days, 1 month, 3 months and 6 months post-treatment. Microbiological eradication rate (in the microbiologically assessable population evaluated according to study protocol) was 82/98 (83.7%) [74.8, 90.4] at 1 month and continued eradication rate 52/57 (91.2%) [80.7, 97.1] at 6 months post-treatment. Comparison of 4 classification schemes in the evaluation of the eradication rate – including the method described in the study protocol and 3 schemes used in previous investigations – showed similar results. Thus, it can be concluded that the present investigation is well comparable in methods and results to previous studies.

Levofloxacin was well tolerated. Four patients (3.4%) discontinued therapy for adverse event and 15 patients (12.8%) experienced at least one adverse event (mainly gastrointestinal (n = 3) and musculoskeletal and connective tissue disorders (n = 3)) during the treatment period and the 7 consecutive days.

Conclusions: Levofloxacin 500 mg once daily given orally for 28 days is clinically and microbiologically effective in the treatment of chronic bacterial prostatitis and is well tolerated.

P1541 Impact of *Enterococcus* ssp. on mortality and morbidity of patients with perforation of the small and large bowel. Retrospective analysis on 473 patients

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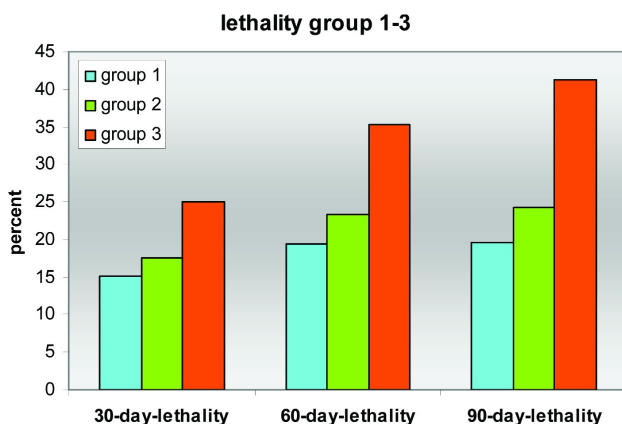
Objectives: Among other bacteria *Enterococcus* ssp. are frequently detected in abdominal fluid after bowel perforation, as well as after major abdominal surgery. The commonly administered antibiotics are not or just partially effective against *Enterococcus* ssp. The need for a specific treatment of *Enterococcus* ssp. is discussed controversely. Agents that are effective ex-vivo penetrate tissue insufficiently or are very expensive. Based on this background and the growing number of vancomycine resistant Enterococci there is demand to identify patients after abdominal surgery who could benefit from therapy.

Methods: We included 473 patients with intestinal perforation. The patients were divided into three different groups, 1: *Enterococcus* ssp. not detected, no therapy, 2: *Enterococcus* ssp. detected, no therapy, and 3: *Enterococcus* ssp. detected, specific therapy. We compared the groups

and analysed how far age, sex, immunosuppression, and detection of *Enterococcus* ssp. influence the course of the disease. Primary endpoint of the study was lethality. Secondary endpoints were morbidity, LOS-ICU and LOS-Hospital. The severity of the disease was assessed using the SOFA-Score (Sepsis-related Organ Failure Assessment).

Results: 90-day-mortality was significantly higher in group 3 compared with group 1 and 2 ($p < 0.001$, graphic 1). There was no difference between group 1 and group 2. LOS-ICU, LOS-Hospital and SOFA-score were significantly higher in group 3 than in group 1 or 2. Age was the only independent factor regarding the course of the disease.

Conclusion: *Enterococcus* ssp. detected in peritoneal fluid after major abdominal surgery are often due to colonisation and do not warrant specific treatment. Nevertheless, Enterococci occurring in peritoneal fluid become increasingly relevant in critically ill patients, as they can worsen the prognosis. Therefore, in critically ill and immunocompromised patients specific treatment of *Enterococcus* ssp. must be considered.



Graphic 1.

Graphic: Statistically significant difference between lethality in group 1 and 2 compared group 3 (30-day: $n < 0.05$; 60-day: $n < 0.01$ 90-day: $n < 0.001$). No difference between group 1 compared with group 2 ($n < 0.3$).

P1542 Efficacy of clindamycin in bacterial vaginosis and risk factors

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Introduction: Bacterial vaginosis (BV) is a common mucocutaneous infection in sexually active women and is caused usually by *Gardnerella vaginalis* overgrowth in vagina due to new infection (i) or normal unbalance (ii) or impairment of colonisation resistance by local or systemic antibiotic (iii), or cleansing during surgery by local antiseptics or after labour due to disinfection procedures in birth ways (iv).

Patients and Methods: The aim of this study was to assess efficacy and safety of clindamycin vaginal cream in therapy of BV in a multicentre study. 3159 patients with BV were enrolled – inclusion criteria was vaginal discharge. Culture was performed in all 3159 women, risk factors such as preganancy, lactation, sexual intercourse within last 3 days, receipt of systemic or local antibiotics or/and antifungal drugs within 7 days, surgery in vaginal or perineal region, etc. were analysed. Lenght and efficacy of therapy and adverse reaction were evaluated. Those who failed after therapy were compared in an univariate analysis with those who were cured using chi square test with Mentel Hanzsel mofification and Yates correction (EPI INFO statistical package).

Results: From 3159 evaluable cases, 2958 (94.3%) cases were cured and 181 (5.9%) were improved, only 20 patients failed (0.7%). Most common risk factors were pregnancy (391 cases, 14.7%), prior local antibiotic therapy (501 cases, 16.5%), prior STD (5.5%), lactation (1.9%), systemic ATB therapy (0.9%) or antifungal agents (47 cases, 1.6%). Most commonest culture isolate was, as expected *Gardnerella vaginalis* in 807 cases (26.5%) followed by *Str. agalactiae* (7.1%), *S. aureus* (4.6%), *Bacteroides fragilis* (2.1%), *E. coli* (1.3%), *Candida albicans* (1%). Analysing predictors of failure, pregnancy was significant more frequently observed among failures (25% vs. 12.7%, $P = 0.001$) and *Streptococcus agalactiae* (35% vs. 7.1%, $P = 0.002$) and *C. albicans* (10% vs. 1%, $P = 0.005$) as well as culture negativity ($P = 0.002$) were predictive for inferior outcome.

Conclusions: In conclusio, clindamycin vaginal creme is mostly effective, safe in the treatment of bacterial vaginosis in health pregnant and non-pregnant women.

P1544 Microbiological effects of a triclosan dentifrice and tongue cleaner in the treatment of oral malodour

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Objectives: Global surveys indicate that oral malodour (halitosis) is a widespread condition affecting almost 50% of people at sometime. Oral malodour is frequently associated with bacteria on the dorsal surface of the tongue. The large surface area and unique anatomy of the dorsal tongue including fissures and papillae facilitate the growth of halitosis-associated microorganisms. This study assessed the effect of treatment for oral malodour on the microflora of the dorsal surface of the tongue.

Methods: Adults were screened for oral malodour and were selected for participation in the study if they exhibited organoleptic scores $\leqslant 3$ (scale 0–5) as determined by a trained examiner and mouth air sulfur levels $\leqslant 250$ ppb as determined by portable sulfide detector. 15 adults were enrolled in the study after informed consent. Each subject was examined at baseline and after 28 days for; (1) organoleptic assessment; (2) mouth air sulfur levels; (3) tongue coating (scale 0–3); and, (4) tongue microorganisms. A 2 cm2 area was sampled from the dorsal surface of the tongue. Bacterial numbers were assessed by microscopy and the presence of 22 bacterial species/groups was assayed by dot-blot using DNA probes. Following the baseline examination, the subjects were instructed to perform oral hygiene with a triclosan-containing dentifrice and to use a toothbrush with a tongue cleaner.

Results: From the panel of halitosis-associated target bacteria, we observed large numbers of *Peptostreptococcus micros*, *Porphyromonas gingivalis*, *Prevotella intermedia*/*Prevotella loescheii*, *Prevotella melaninogenica*, *Solobacterium moorei* and *Streptococcus* sp. at baseline. After 28 days, all subjects showed clinical improvement as determined by significant reductions in organoleptic scores and mouth air sulfur levels compared to baseline ($p < 0.0001$). Microscopic analysis showed a greater than 70% reduction in the numbers of microorganisms ($p < 0.001$) while dot-blot assays showed significant reductions in *Enterococcus faecalis* ($p < 0.003$), *Neisseria* sp. ($p < 0.008$), *P. micros* ($p < 0.0007$), *P. melaninogenica* ($p < 0.02$), *P. gingivalis* ($p < 0.0001$), *S. moorei* ($p < 0.04$), and Streptococci sp ($p < 0.03$) from the dorsal tongue surface.

Conclusions: All subjects with oral malodour demonstrated significant improvements in both clinical and microbiological parameters following use of the triclosan-containing dentifrice and toothbrush with a tongue cleaner.

P1545 Single-dose azithromycin vs 7 days doxycycline for *Chlamydia trachomatis* urethritis in men: a systematic review and meta-analysis of randomised clinical trials

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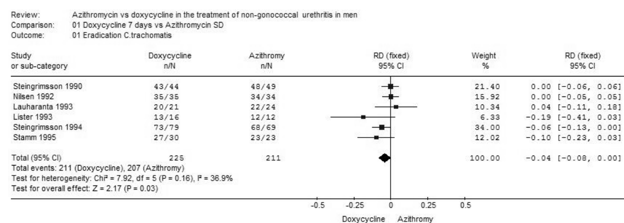
Objectives: Both azithromycin and doxycycline are recommended as first-line treatment for *Chlamydia trachomatis* urethritis (CTU) in international and national STD guidelines. Therefore comparative efficacy and safety of single dose azithromycin (SD) vs 7 days doxycycline is very important question. A systematic review (SR) comparing these antibiotics has not demonstrated significant clinical or microbiological efficacy rate after azithromycin vs doxycycline based

therapy (Chuen-Yen Lau e.a, 2002). However as male with urethritis only and female with cervicitis mainly were combined in mentioned SR. The goal of our study was to perform a SR and metaanalysis (MA) for evaluating the efficacy and safety of azithromycin versus doxycycline in less heterogeneous population – only in men with CTU.

Methods: We searched the Cochrane Central Register of Controlled Trials (CENTRAL, in The Cochrane Library Issue 3, 2007), MEDLINE (1966 – September 2007), EMBASE (1988 – September 2007), reference lists of articles and abstracts from conference proceedings without language restriction. Reference lists of urology, infectious diseases and STD textbooks, review articles and relevant studies. Randomised and quasi-randomised controlled trials comparing single dose azithromycin vs 7 days doxycycline in men (>18 years) with uncomplicated CTU were selected. Two reviewers independently assessed trials quality and extracted data. Statistical analyses were performed using the random effects model and the results expressed as relative risk (RR) for dichotomous outcomes with 95% confidence intervals (CI).

Results: We identified 6 studies met the inclusion criteria, 436 patients were evaluated for microbial cure. Microbiological eradication rates were 93.8% for azithromycin and 98.1% for doxycycline, RR = 0.04, p = 0.03; 95% CI = 0.08–0.00.



Conclusion: Doxycycline for 7 days as compared with SD azithromycin has small but statistically significant superiority in eradication rate of *C. trachomatis* in men with CTU. Clinical importance of this difference may not be very significant but in further studies we should consider possible data heterogeneity when combined studies compared antimicrobials for *C. trachomatis* infections in patients with different nosologies (e.a. urethritis and cervicitis).

P1546 Prophylaxis of respiratory tract invasive fungal infections with aerosolised amphotericin-B deoxycholate in allogeneic haematopoietic stem cell transplant recipients

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Objectives: Prophylaxis of fungal infections (IFIs) is still a major challenge in patients (pts) undergoing stem cell transplantation (HSCT). The aim of this retrospective study was to evaluate the impact of prophylactic aerosolised amphotericin-B deoxycholate (d-AmB) on respiratory tract IFIs in a homogeneous cohort of allogeneic HSCT pts, transplanted at our institution.

Methods: Since 1999, 87 consecutive pts were transplanted from matched related (N=65) or unrelated donor (N=22). Analysis was performed on 78 evaluable pts, in order to monitor the prevalence of possible, probable, proven IFIs of the respiratory tract within 40 days after HSCT. Conventional antifungal prophylaxis was based on the association of IV/PO fluconazole (400 mg/d), plus aerosolised d-AmB (15 mg bid) in 58 out of 78 cases (74%). All the pts were screened before transplant and followed-up thereafter with CT or x-rays (paranasal sinuses, thorax), galactomannan antigenaemia and surveillance swabs. Chi square test was performed to evaluate correlations between variables.

Results: Aerosolised d-AmB was administered to 74 pts for a median time of 16 days (range 2–45). Prolonged administration was not associated with increased life-threatening bacterial infections, nor severe adverse events were observed; only a pt developed moderate bronchial spasm. In 13 pts, aerosolised d-AmB was delivered for less than 7 days, due to worsened clinical conditions, or poor compliance. In this group, proven IFIs were diagnosed in 2 pts (1 mucormycosis and 1 fusariosis), possible aspergillosis in one and probable aspergillosis in another one. A shortened administration (<7 days) of aerosolised d-AmB was associated with an increased risk of IFIs (p = 0.004). Overall, 94% of pts did not experience IFIs and none died due to IFIs. Nine pts had a pre-transplant nasal swab positive for *Aspergillus* spp., and 8 of them received aerosolised d-AmB; their subsequent surveillance swabs proved negative. The only patient with positive swab who did not undergo aerosolised d-AmB due to bronchial spasm developed a possible aspergillosis.

Conclusions: Prolonged aerosolised d-AmB seems to play a role in preventing respiratory tract IFIs, but a randomised controlled trial is warranted, in order to verify the impact of such prophylactic regimen in this setting.

P1547 Is cotrimoxazole prophylaxis still reliable for the prevention of nocardiosis?

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Background: Clinical and microbiological spectrum of nocardiosis has changed recently due to the widespread use of cotrimoxazole (SXT) prophylaxis, the emergence of new types of immunosuppressed patients (pts) and the improved identification of the isolates by means of molecular techniques.

Objective: To establish the incidence and the microbiological and clinical characteristics of nocardiosis in a general hospital over the last 12 years.

Methods: Review of the clinical records of all pts with *Nocardia* spp. cultured from a clinical specimen between 1995 and 2006. *Nocardia* isolates were identified with classic procedures and by hsp65 PCR-RFLP. MICs of SXT, minocycline, imipenem, meropenem, linezolid and amikacin were determined following the CLSI microdilution method.

Results: *Nocardia* was recovered from 43 patients. Six were considered colonised. All had severe underlying pulmonary pathology and were treated with antimicrobials (6) or corticosteroids (4). Colonising species corresponded to *N. farcinica*, *N. nova* and *N. asteroides*.

Invasive nocardiosis was diagnosed in 37 pts; 86.5% were men and mean age was 55.8±17.3 y.o. The most common underlying condition was HIV infection (10 pts; 27%), followed by neoplasic disease (4 pts; 21.6%), COPD (8 pts; 21.6%), autoimmune diseases (8 pts; 21.6%) and solid organ transplantation (7; 18.9%). Most important risk factor was corticosteroids administration (23 pts; 62.2%). Nocardiosis was disseminated in 5 cases (13.5%), pulmonary in 70.3%, cutaneous in 8.1%, affected the CNS in 5.4%, and caused otomastoiditis in 1 case (2.7%).

Species were: *N. asteroides* VI 45.9%, *N. farcinica* 24.3%, *N. otitidis-caviarum* 10.8% and *N. nova* 5.4%. Antimicrobial resistance rates were: SXT 8.3%, meropenem 16.6%, imipenem 11.2%, mynocicline, linezolid and amikacin 0%. Nocardiosis occurred while the patients were on SXT prophylaxis in 11 pts (29.7%) and the strains isolated in those pts were susceptible to SXT in 33 (91.6%) and resistant in 3 (8.3%)(SXT susceptibility not known in one strain). Overall, 13 pts died (21.6%) and related-mortality was 21.6% (8 pts).

Conclusions: Our results show important changes occurring in nocardiosis in recent years. HIV infection is presently the most common underlying condition and species different from *N. asteroides* account for more than half of the infections. Moreover, cotrimoxazole prophylaxis should not be relied upon as universally protective.

P1548 Oral moxifloxacin versus ciprofloxacin plus amoxicillin/clavulanic acid for low-risk febrile neutropenia – A prospective, double-blind, randomised, multicentre EORTC-Infectious Diseases Group (IDG) Trial (protocol 46001, IDG trial XV)

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Background: Oral antibiotic therapy with ciprofloxacin plus amoxicillin/clavulanic acid (C+A) is as efficacious as parenteral therapy in cancer patients with low-risk febrile neutropenia (FN). Outpatient management may be an option provided that patients are carefully selected. Moxifloxacin (Moxi) is a new fluoroquinolone with activity against streptococci and staphylococci that is superior to that of ciprofloxacin. Moxi given as single daily oral dose might be a suitable option for empirical therapy of FN.
Methods: The EORTC-IDG performed a prospective, double-blind, randomised, multicentre trial comparing the efficacy and safety of oral Moxi (1 x 400 mg) and C+A (2 x 750 mg + 2 x 1000 mg). Inclusion criteria were FN, a MASCC score >20 (predicting a low risk of medical complications), and ability to swallow. A single initial dose of parenteral empirical antibiotic therapy prior to enrolment was allowed. Patients could be discharged according to predefined medical and non-medical criteria. The trial was designed to show equivalence (<10% difference in response rates) of both regimens in terms of efficacy.
Results: The trial was terminated after randomisation of 341 patients of whom most (66%) were outpatients at fever onset, half had solid tumor, only few (28%) had received an initial intravenous antibiotic dose prior to study drug therapy initiation, and 333 were eligible and evaluated in an intention-to-treat (ITT) analysis. Most of these patients (59%) had fever without documented infection, and only 12% had bloodstream infection (44 pathogens, 22 gram-positive, 22 gram-negative). Therapy success in the ITT population was observed in 136/169 patients given Moxi (80%) and in 134/164 patients given C+A (82%), a difference of −1% (95% confidence interval, −9% to 8%) which supports equivalence in efficacy of the two arms. Study drug treatment duration was 7 days in each arm. Comparable success rates in the two arms were observed across different subgroups, and similar proportions of patients in the two arms were discharged over time. There were slightly fewer patients in the Moxi arm who developed superinfection (7% vs 14%, p = 0.05). The rates of patients with at least one adverse event (44% vs 52%, p = 0.13), or serious adverse events (4% vs 7%, p = 0.15) were similar. Two patients in each arm died within 30 days following randomisation.
Conclusions: Therapy with Moxi at single daily doses of 400 mg orally is as effective for low-risk FN as C+A.

Tuberculosis: epidemiological and clinical studies

P1549 Evaluation of rapid genotypic assays for the detection of MDR-TB strains from a Cape Town hospital

J.C. Evans, M. Stead, M. Nicol, H. Segal (Cape Town, ZA)

Objectives: An estimated one third of the world's population is infected with *Mycobacterium tuberculosis*, with 350 cases per 100 000 population in sub-Saharan Africa alone. The problem is further compounded by the emergence of multidrug resistant-*M. tuberculosis* (MDR-TB), resistant to rifampicin (RIF) and isoniazid (INH). RIF resistance arises predominantly due to amino acid substitutions in rpoB, specifically at codons 531, 526 and 516. Up to 80% of INH resistance is due to mutations in KatG (S315T) and inhA (C-15T). Detection of these mutations often form the basis of rapid diagnostic assays for the detection of MDR-TB.
The aim of this study was to determine the frequency of resistance-determinants in MDR-TB strains from Groote Schuur Hospital to evaluate the efficacy of the GenoType® MTBDRplus assay. Genotyping was carried out to determine whether particular lineages are associated with emergence of MDR-TB.
Methods: The GenoType® MTBDRplus assay was evaluated on isolates identified as MDR (95), INH mono-resistant (67) and RIF mono-resistant (11) by drug susceptibility testing.
The presence of KatG S315T and inhA C-15T, identified using the GenoType® MTBDRplus assay, was confirmed by multiplex allele-specific (MAS) PCR assays and sequencing.
All isolates were genotyped using spoligotyping and MIRU-VNTR.
Results: Of the 46 MDR and 10 RIF mono-resistant isolates, 44 and 6, respectively, were identified as RIF resistant using the GenoType® MTBDRplus assay. This assay detected INH resistance in 41/46 MDR isolates and 25/44 INH mono-resistant strains.
MAS-PCR assays confirmed the presence of the same INH resistance-determinants in the 41 MDR isolates, and detected INH resistance in a further 8 INH mono-resistant strains. MAS-PCR failed to detect an inhA C-15T mutation in one isolate that was detected by the GenoType® MTBDRplus assay and confirmed by sequencing.
Spoligotyping indicated an association between the W-Beijing lineage and MDR strains. RIF mono-resistant strains (8/11) were of the LAM3/F11 lineage and the same MIRU-VNTR cluster.
Conclusion: The GenoType® MTBDRplus assay was successful at detecting RIF resistance. However, 11% of MDR and 43% of INH mono-resistant strains were indicated as being INH susceptible, suggesting that this assay should be interpreted with caution in this setting.
That all the RIF mono-resistant strains cluster together may suggest the presence of a dominant clone that could emerge as an MDR strain following conventional TB treatment.

P1550 Detection of mutations conferring rifampicin resistance in *Mycobacterium tuberculosis* using real-time PCR and high-resolution melt analysis

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Objectives: High-resolution melt (HRM) and the new LCGreen dyes can be used to detect single nucleotide polymorphisms in amplified PCR products. Sequence changes produce a Tm shift and difference in the melting curve shape compared with the wild-type. Ninety-five percent of rifampicin resistance mutations in *Mycobacterium tuberculosis* (TB) are located in an 81bp rifampicin-resistant determining region (RRDR) of the rpoB gene. The aim of this study was to design an assay for the rapid detection of rifampicin resistance. Rapid detection of resisatnce is important clinically as appropriate treatment can be commenced immediately resulting in a more favourable outcome for the patient.
Methods: A 411bp fragment of the rpoB gene including the RRDR was amplified using real-time PCR followed immediately by HRM to determine whether mutations were present.
Results: Of the isolates tested, 22/24 rifampicin-sensitive isolates and 15/15 rifampicin-resistant isolates were correctly identified. Of the resistant isolates, HRM analysis differentiated between three of the most common RRDR mutations (S531L, H526Y and H526D).
Conclusion: This assay is an inexpensive method for the rapid detection (<3 hrs) of rifampicin-resistance in TB.

P1551 Use of a multi-locus approach to determine the phylogenetic position of 5 unidentified *Mycobacterium* strains isolated from clinical samples

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Molecular sequencing and genomic studies have accumulated evidence for the definition of species concept. 16SrRNA is inadequate for a complete resolution of species of Bacteria. The Multilocus Approach appears to be useful for the classification of Bacteria. We wanted to perform a MLSA study on the *Mycobacterium* genes and applied this method to strain remained unidentified by the 16S rRNA sequencing.

Methods: we worked on the whole type strain collection of the genus *Mycobacterium*, sequenced 7 genes (16S rRNA, hsp65, rpoB, sodA, smpB, tuf, ssrA) and created a phylogeny on the concatenated sequence by Maximum Likelihood method (better tolerance of missing data). We submitted the 7-genes concatenated sequence of each unknown strain and reanalysed the global alignment by PHYML.

Results: the phylogeny constructed by ML was the first one on this genus and resulted in the usual topology with early separation of the rapidly growing group and of the slowly growing group. Usual pathogens clusters are well grouped. Applying the analysis on the 5 clinical isolates that remained unidentified, the MLSA approach confirmed the up-to-now undescribed status of strains.

Conclusion: As recommended by the Stackebrandt Committee, the MLSA approach is a useful tool for better resolving species and necessary for description of new species. And even if this approach still remains too expensive for a routine use, it might constitute an interesting trend for bacterial identification when the resolving power of the 16S rRNA is insufficient.

P1552 DNA fingerprinting of *Mycobacterium tuberculosis* isolates in the UK: analysis of strains originating from the Indian sub-continent

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Objectives: From 2003 onwards, all isolates of *M. tuberculosis* in the UK Midlands have been analysed by MIRU-VNTR typing. The predominant group of patients presenting with TB in the Midlands originate from the Indian Sub-Continent (ISC) and it has been shown that *M. tuberculosis* strain types are closely related to host global origin. To detect strain origin we used OriginsInfo software which analyses given and family names and then assigns a global origin. This software has been recently developed to analyse names originating from India. The aim of this study was to examine and begin to define the prevalent strains originating from the ISC.

Methods: From 2003–2007, 4,226 isolates identified as *M. tuberculosis* by the Hain LifeScience GenoType MTBC assay and phenotypic methods were analysed by MIRU-VNTR typing using 3 ETR loci and 12 MIRU loci analysed on a Transgenomic WAVE system. Patient ethnic origin was assigned based on names using OriginsInfo software. Significant associations ($OR > 1.00$, $p < 0.05$) between clades and patient populations were identified using odds ratios.

Results: OriginsInfo software identified 2,456/4,226 (58.1%) isolates originating from the ISC. Of 396 VNTR clades present, there were 17 predominant VNTR clades significantly associated with the ISC, ranging from 6–656 isolates in size. The largest distinct clades were VNTR 42235 (656, 15.5%), 42234 (189, 4.5%), and 61464 (44, 1.0%). The first two clades are members of the CAS family. The largest clade (42235) was shown to be significantly associated with patients originating from Bangladesh, India, and Pakistan with OriginsInfo associating the 42235 clade with 9 states across India and significantly with Gujarat, Daman and Diu, Maharashtra, Goa, the Punjab, and West Bengal. The most prevalent MIRU-VNTR profiles were 42235 225425173533 (100, 2.4%), 42235 226425153533 (45, 1.1%), and 42234 226425173423 (41, 1.0%). The most prevalent MIRU-VNTR profile was associated with 7 states and significantly associated with patients originating from the Punjab.

Conclusion: There are several prevalent clades of TB present in the UK Midlands that are significantly associated with the ISC. The most predominant VNTR clade (42235) and MIRU profile (225425173533) are present in many regions of India. Combination of molecular data with global anthropological data provided by OriginsInfo provides a powerful analysis and insight into the origin of *M. tuberculosis* strains.

P1553 MIRU-VNTR genotyping of *Mycobacterium tuberculosis* strains from central region of Russia

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Objectives: The Russian Federation is one of the world's areas most affected by a high incidence of tuberculosis (TB). Although TB morbidity decreased slightly in recent years, the situation remains alarming due to a major increase in the incidence and prevalence of multidrug-resistant TB (MDR-TB). The purposes of this study were to evaluate epidemiological diversity and MDR spreading among *M. tuberculosis* strains, circulated in the Central region of Russian Federation.

Methods: *M. tuberculosis* strains ($n = 35$) from sputum of epidemiologically unrelated TB patients from Moscow were selected for study. Species identification and drug susceptibility testing to main antituberculosis drugs – rifampicin (RIF) and isoniazid (INH) – were performed according to the WHO recommended protocols. Standard procedure of MIRU-VNTR typing was carried out as described previously. For detection of specific SNPs causing drug resistance minisequencing reaction followed by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) of the reaction products was used.

Results: Among 65 *M. tuberculosis* strains 33 (50.8%) were identified as MDR (i.e. resistant to both RIF and INH), 19 (29.2%) were susceptible to RIF and INH, and 13 (20.0%) were resistant to INH only. Mutations in the RRDR of the rpoB gene were detected in all RIF-resistant strains. The Ser531Leu substitution was prevalent among them (24/33, 72.7%). Aberrations in the Ser315 codon of katG and/or in the inhA promoter region were found in 41 (89.1%) of 46 INH-resistant strains. Most of them (33/46, 71.7%) had the mutation Ser315Thr. Thirty five different MIRU-VNTR profiles were identified, including the 27 unique ones. 8 clusters (from 2 to 11 strains in each one) enclosed the 38 strains. Mutations associated with the MDR-phenotype were found more frequently in strains grouped in clusters (23/38; 60.5%), than for strains with unique MIRU-VNTR profiles (10/27; 37.1%). (Chi2 for trend 4.4; $P < 0.05$).

Conclusion: Those strains grouped into the main VNTR clusters and had the MIRU profiles 223325153533, 223325173533 or 223425173533 are associated with MDR-phenotype. It is possible that the spreading of such strains is related with secondary-acquired resistance and plays a significant role in increasing of MDR TB in the Central region of Russia.

P1554 Immigration and tuberculosis evolution in Catalonia central region. 10 years (1996–2005)

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Introduction: Immigrants from countries with a high tuberculosis (TB) prevalence may affect tuberculosis control in low incidence countries because there is a gradient of risk of disease among populations. The foreign-born, have emerged as the largest single component of the burden of tuberculosis in most industrialised countries.

Methods: Two periods cohort study: from 1996 to 2000 and from 2001 to 2005.TB notified cases from Catalonia Central Region-CCR-(6577 km2 and 1528144 inhabitants) has been included.

Proportion of immigrants – foreign-born arrived to CCR – were obtained.
Indicators of TB control performance as proportion of tuberculosis cases with unsuccesful treatment outcome (UTO) and proportion of contact tracing (CT) within sputum smear positive patients(AFB+) were estimated as follows:

UTO: defaults, plus treatment failures, plus transfers/total patients with pulmonary tuberculosis. Goal <10% (IUATLD/WHO)

CT in AFB+ goal: over 85%.

Overall and spanish population TB incidence per 100.000 inhabitants and its average annual decline (AAD) were calculated.

Results: Immigrants proportion increased from 1.2% in 1996 to 1.5% in 2000 and from 2.9% in 2001 to 8.5% in 2005. 95% foreign-borns came from countries with 50–99 TB cases /100,000 hab.

1996–2000 period:

1530 new TB cases were notified (6% of whom were immigrants).

UTO: 8.8% in spanish vs 23.2% in immigrants (p < 0001).

CT: 88.5% in spanish vs 40.5% in immigrants (p < 0001).

Overall TB incidence: 28.3 in 1996 and 18.9 in 2000, AAD 9.6%.

Spanish population incidence rates decreased from 27.5 in 1996 to 16.7 in 2000; 11.2% AAD.

2001–2005 period:

1352 new TB cases were notified (immigrants, 21.8%).

UTO: 7.6% spanish vs 28.9% immigrants (p < 0001).

CT: 91% spanish vs 62.2% immigrants (p < 0001).

Overall TB incidence decreased from 20.8 in 2001 to 18.4 in 2005, 3% AAD.

Spanish population incidence rates decreased from 18.8 in 2001 to 14.8 in 2005; 5.8% AAD.

Conclusions: Immigrant population shows poorly tuberculosis control performance results (UTO > 10% and low proportion of contact tracing). Notification data indicate that the decline has slowed down after first period despite spanish population tuberculosis control measures has been effective (UTO < 10%). As TB incidence decrise in spanish, increase the proportion of immigrants among tuberculosis cases.

P1555 Epidemiological and clinical features of 85 patients with tuberculosis at a teaching hospital in Italy

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Background: A previous similar study performed at the same institution (Hospital of Pisa) during the 1996–2000 period showed a TB incidence ranging from 8.5 to 6.8 (N/100.000), a rate of extrapulmonary TB of 24.5% and a 3.8% frequency of isolates resistant to ⩽2 drugs.

Aim of the study and methods: In order to describe current epidemiologic and clinical features of patients with TB identified in the hospital of Pisa (Italy), a retrospective study of all cases of TB notified to the Local Public Health Service during January 2004 – December 2006 was performed. The diagnosis of TB was made following the criteria of the WHO.

Results: A total of 85 patients (M 39, F 46) affected by TB were identified. Diagnosis was microbiologically proved in 81 patients. Thirty-five patients were immigrants (mostly from East-Europe and South-East Asia). The incidence of TB (N/100.000) was 9.5, 8.0 and 7.0 in 2004, 2005 and 2006, respectively. 66.5% of patiens had pulmonary TB; 33.5% of patients had extrapulmonary or mixed TB.

Extrapulmonary TB was more frequent in immigrant patients (42.8%) than in italian ones (22%). Five patients were presenting advanced HIV infection. Microscopic examination for acid fast bacilli in sputum or bronchial secretion resulted negative in 67.3% of colture proved pulmonary TB.

7% of the isolated strains of M.tubercolosis were resistant to ⩽2 drugs.

Conclusions: The incidence of TB observed in the present study seems to be similar to that observed at our institution during the 1996–2000 period. The rate of extrapulmonary TB shows a trend to increase expecially in immigrant patients.

The isolation rate of resistant strains to drugs of *M. tuberculosis* in our geographical area shows an increasing trend.

P1557 Estimation of tuberculin status among school children on a Greek island

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Objectives: The aim of the study was to estimate the tuberculin status among first class school children in the Island of Corfu.

Methods: In the time period from 1991–2005 tuberculin skin tests were performed on primary school children at 6 years of age. To all children qualified medical and nursing personnel administered intra-dermal Mantoux test with 0.1 ml PPD. The site of the antigen injection was examined for occurrence of indurations after 48–72 hours. Children with positive test results (indurations of 10 mm and more) were regarded as infected with *M. tuberculosis* and were referred to a paediatric clinic for further examination and investigation. All children who had a response of less than 10 mm (test negative) received a BCG vaccination. Children born in Albania and showing a response of more than 10 mm were also considered as positive and referred to further investigation, although this fact could issue from previous BCG-vaccination at birth in the origin country.

Results: During the fourteen year study period a total of 9072 children were screened for *M. tuberculosis* infection. A total of 63 of the screened children were found to be positive giving an average prevalence rate of 0.7%. Comparing the two seven-year periods (1991–1998 and 1999–2005) the average prevalence among the primary school children showed a significant decrease from 1.1% to 0.1%.

Conclusions: The prevalence rates of infection among children at school enrollment demonstrate a falling tendency, supporting the impression that satisfactory progress has been achieved in control of tuberculosis. Actually we were expecting a slightly increase in the prevalence of positive tuberculin skin tests since 1996, when children of immigrants coming from TB endemic areas were enrolled in the primary school. On the contrary, we noticed a further reduction of the prevalence rates. This fact seems to be justified by vigilant control programmes for the immigrants realised by the local public services. It is important to retain low rates of tuberculosis in remote areas and especially in border areas with a high immigration. Therefore, good organised local control and surveillance systems are essential in order to achieve effective disease control and prevention of tuberculosis.

P1558 Tuberculosis in a Canadian paediatric tertiary care hospital: identification of two high-risk communities

M. Clark, C. Hui (Ottawa, CA)

Objectives: The catchment area of the Children's Hospital of Eastern Ontario (CHEO) includes three areas: eastern Ontario; western Quebec; and Baffin Island. The latter is part of the Canadian arctic, accounting for 1% of the hospital's total catchment population. Patients from this region have been transferred to CHEO for care since 1998. Objectives of this study were: to describe the origin of tuberculosis (TB) patients at CHEO; and to describe their clinical characteristics.

Methods: All charts with a discharge diagnosis of TB since 1998 were reviewed. Patients with a positive culture were considered confirmed TB cases. "Probable" cases included patients with radiological findings consistent with TB, absence of a more likely diagnosis, improvement on antitubercular therapy, and one or more of the following: a positive skin test; contact with a respiratory, culture-positive case; or a smear positive for acid-fast bacilli.

Results: Twenty-seven charts were reviewed, of which 19 met our case definition. Cases ranged in age from one to 17 years. Ten (53%) were female. Seven out of 19 total cases (37%) were Canadian-born, Inuit children from Baffin Island. Seven cases resided in Ontario, while four lived in Quebec and one in England. There were seven foreign-born children residing in Ontario or Quebec, all of them from African countries, including five from Somalia.

Main diagnostic sites included: lung (10); pleura (3); central nervous system (CNS) (3); abdomen (1); spine (1); and superficial lymph nodes (1). Two girls aged 12 years developed cavitary disease. Complications included oesophageal erosion, corneal ulceration, empyema, and psoas abscess. CNS disease occurred in two children from Baffin Island, and an HIV-infected child from Rwanda. One of the two from Baffin Island died, while the other was left with a hemiparesis. The child from Rwanda did well after excision of a brain abscess.

Conclusion: Here we identify two communities which make up a small proportion of the CHEO catchment population, and contribute the vast majority of the hospital's TB patients. We also show that TB in children can present in many different ways, and cause a number of complications. TB in children is an indicator of ongoing transmission in a community.

Together, children from Baffin Island and Africa account for 74% of our TB admissions. The problem in Baffin Island is the result of a home-grown epidemic, and cannot be attributed to importation from abroad.

P1559 Multidrug and extensive drug-resistant tuberculosis in Lisbon, Portugal: molecular epidemiology and resistance mechanisms

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Objectives: Portugal has one of the highest tuberculosis notification rates of the European Union. In 2003, Portugal reported a tuberculosis incidence rate of 36.4/100 000 and a primary multidrug-resistant tuberculosis incidence of 1.5%. However, in Lisbon Health Region only, we have identified 116 MDR-TB strains in 1200 *Mycobacterium tuberculosis* isolated strains. Therefore, our objectives were to assess the multidrug and extensively-drug resistant tuberculosis situation in Lisbon, through a molecular epidemiological approach combined with mutational analysis.
Methods: We have analysed 101 multidrug-resistant strains collected from several hospital units across Lisbon Health Region in the years of 2003 and 2004. All isolates were typed by Mycobacterial Interspersed Repetitive Units (MIRU) and, screened for mutations conferring resistance to isoniazid, rifampicin and pyrazinamide accordingly to their resistance pattern. Drug susceptibility tests for second-line drugs were also performed.
Results: We have verified that the majority of the studied isolates belong to a family of strains, identified several years ago, designated as family Lisboa. Despite the high degree of clustering, no significant outbreak was detected. The use of a mutational analysis has allowed the discrimination between isolates of the same cluster.
We have also determined the most prevalent mutations associated with resistance to isoniazid, rifampicin and pyrazinamide. Mutations associated with resistance to isoniazid, rifampicin and pyrazinamide occurred mainly in the regulatory region of the mabA-inhA operon, codon 531 of the rpoB gene, and codons 120 and 125 of the pncA gene, respectively. The prevalence of extensively drug-resistant tuberculosis among the studied isolates was found to be approximately 54%.
Conclusion: We conclude that the majority multidrug strains circulating in Lisbon Health Region belong to family Lisboa, a family involved in an outbreak almost 10 years ago. If measures are not taken to contain such strains, they will continue to circulate and cause more multidrug resistant tuberculosis cases and ultimately, developing into extensively drug-resistant strains, as it is already happening.
The most prevalent mutations associated with drug resistance were also determined. Such knowledge may be used to develop a locally adapted molecular diagnostic test.

P1560 Decrease in incidence of tuberculosis in Madrid is associated to declining rates of recent transmission: an epidemiological and molecular study between 1997 and 2004

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Objectives: Longitudinal studies using molecular and conventional epidemiological methods are necessary to understand whether changes in incidence rates in a particular geographic area can be caused by changes in ongoing transmission. The aim of this observational study was to determine to what extent TB trends in Madrid from 1997 to 2004 were determined by changes in recent transmission of disease.
Methods: Two prospective population-based molecular and epidemiological studies of patients diagnosed with TB were conducted in three urban districts of Madrid during two different periods. The first one was performed between 1997–1999 (population: 455,050, 4% immigrants) and the second between 2002–2004 in the same geographic area (population: 488,518, 14.9% immigrants). DNA fingerprinting was performed using IS6110 and spoligotyping.
Results: In the first period the incidence of TB was on average 30.2 cases/100,000, while for the second was 25.7/100,000 ($p<0.001$). The comparison of TB cases from two study periods showed an important increase of foreign-born population from 5.3% to 34% ($p<0.001$), and a decrease of HIV infection from 25.7% to 12.2% ($p<0.001$). In the first period, 95 of 212 patients (44.8%) were grouped in clusters, and in the second, 64 of 201 patients (31.8%). The decrease in the overall case rate was associated to a decrease in the clustered case rate, 7.0 per 100,000 in 1997–1999 to 4.4 in 2002–2004 ($p<0.001$). The non-clustered case rate was 8.6 in 1997–1999 and 9.3 in 2002–2004 ($p=0.45$). The overall incidence among Spain-born population decreased from 29.9 to 20.0 ($p<0.001$), and this decrease occurred either in cases attributed to recent transmission ($p<0.001$) and cases attributed to TB reactivation ($p=0.04$). Among the foreign-born population although there was not significant changes in the overall case rate (40.0 vs 58.7), however the non-clustered case rate increased significantly ($p=0.02$). Decrease in clustered case rates were also observed in women ($p=0.02$) and persons younger than 35 years-old ($p=0.02$).
Conclusions: The percentage of recent transmission of TB has decreased from 1997–99 to 2002–04 in Madrid and this change is associated to declining rates of recent transmission occurring mainly in the Spain-born population. The reduction in the proportion of clustered cases was also related to the increase in the number of TB cases among foreigners which could mean the introduction of new strains.

P1561 Is there any difference between non-smoker and smoker tuberculous patients in clinical manifestations and radiographic findings?

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Introduction and Aim: Cigarette smoking and tuberculosis are responsible for a large portion of morbidity and mortality in the developing countries. It has been well documented that smoking increases the risk of tuberculosis (TB). However, a few studies have evaluated the impact of smoking on TB. In order to evaluate the influences of smoking on the clinical manifestations and radiographic findings in smoker tuberculous patients, we conducted this study.
Materials and Methods: From May 2005 to December 2006, two hundred smear positive pulmonary TB patients who referred to Zahedan tuberculosis centre for treatment, identified. Then, we evaluated the influences of smoking on the Clinical and radiograohic manifestations of TB.
Results: A total of 200 TB patients, including 100 (50%) who had ever smoked, were studied. There was no significant difference in age between smokers and non-smokers. Smokers more likely to have pulmonary underlying diseases such as chronic bronchitis and asthma and a duration of symptoms >three months, and less likely to have extra-pulmonary involvement. Radiographically, more smokers with pulmonary tuberculosis (PTB) presented with cavitation, miliary lesions, multiple nodules or masses, and infiltrations in two lungs than non-smokers. Also, there was a significant delay in sputum smear conversion time in smoker patients with pulmonary tuberculosis at 2, 4 and 6 months of treatment ($P<0.05$).
Conclusion: Our results showed that smoking is significantly associated with clinical and radiographic manifestations of PTB and a delay in sputum smear conversion time.

P1562 Tuberculin response in indigenous Warao children in Venezuela; BCG vaccination doesn't affect the positive rates

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Background: The in vitro interferon-gamma (IFN-gamma) assay has emerged as a more specific alternative to the tuberculin skin test (TST) for the diagnosis of latent tuberculosis (TB) infection in BCG vaccinated persons. However, to justify the introduction of this new and expensive test, studies in low-income countries must show that BCG vaccination significantly affects the TST results.

Objective: To determine the specificity of the TST is diminished in BCG vaccinated children from a high TB incidence, indigenous Warao population in Venezuela.
Methods: 1479 children, 1–15 years of age, from different Warao communities were tested using 2 TU RT 23 tuberculin. Reactions were measured at 48 hours. The presence of the BCG scar and age of the child was recorded. A reaction of 10 mm or more was considered as positive. The Kruskal-Wallis and the Pearson test were conducted for the analysis of the results.
Results: 1088 children in this study had a BCG scar and 391 children did not. The age distribution in both groups was not significantly different. A positive TST was found in 10.8% of the BCG vaccinated and 8.5% of the non-vaccinated children, a difference that was not statistically significant. In both groups a positive TST was strongly related with the age of the child and there was no significant difference of TST positive in the same age groups of the vaccinated and non-vaccinated children.
Conclusion: In this study BCG vaccination does not significantly affect the results of the TST test and is cheaper and easier to perform than the interferon gamma test. The TST test is still a valuable tool for detecting tuberculosis infection, especially in this rural setting. In a future study we will determine whether there is a significant difference in the sensitivity of the two tests for the Warao population.

P1563 Pooled analysis of extra-pulmonary tuberculosis in Turkey: a review of 2,168 patients

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Objectives: In this study it was aimed to review the published extrapulmonary tuberculosis literature by pooled analysis method.
Methods: To find out the published series in last fifteen years three national databases (Ulakbim Turkish Medical Literature database, http://www.turkishmedline.com, http://medline.pleksus.com.tr) and two international databases [Pubmed and Science Citation Index (SCI)] were searched. Keywords for national databases were ["akciger disi tuberkuloz" or “ektra pulmoner tuberkuloz” or “tuberkuloz plorezi” or “tuberkuloz lenfadenit” or “deri tuberkulozu” or “tuberkuloz perikardit” or “genitouriner sistem tuberkulozu” or “tüberküloz menenjit” or “milier tuberkuloz” or “vertebra tuberkulozu” or “larinks tuberkulozu” or “tuberkuloz peritonit”]. Keywords for Index Medicus and SCI-expanded were [{“extrapulmonary tuberculosis” or “tuberculosis pleurisy” or “tuberculosis lymphadenitis” or “skin tuberculosis” or “tuberculosis pericarditis” or “genitourinary system tuberculosis” or “tuberculosis meningitis” or “miliary tuberculosis” or “vertebral tuberculosis” or “larynx tuberculosis” or “tuberculosis peritonitis”} and Turkey]. Articles i) in which clinical classification (ie. Involved organs) of extrapulonary tuberculosis was not defined, ii) published before 1993, were excluded.

Table. Distribution of the extrapulmonary tuberculosis cases, diagnostic modalities

Clinical diagnosis	Total no. of cases	Female (n)	Male (n)	Age (mean)	ARB(+) (n)	Culture(+) (n)	Diagnosed by biopsy (n)
Meningitis	820	393	427	23.6	112	131	–
Pleura	405	146	259	36.1	23	13	180
Lymphadenitis	357	248	109	34.9	26	23	222
Skin	190	115	75	23.1	8	12	172
Bone-joint	124	65	59	36.5	14	19	51
Miliary	88	40	48	44.5	51	41	–
Genito-urinary system	68	28	40	39.8	21	10	26
Peritonitis	57	31	26	29.4	7	5	41
Pericarditis	11	7	4	47	1	–	3
Larynx	10	–	10	33.1	5	8	6
Other	38	23	15	40	6	7	21
Total*	2168*	1096	1072	–	274*	269*	722*

*Other cases were diagnosed by clinical findings suitable with tuberculosis + response to antituberculosis therapy.

Results: Data for 2168 patients (50.55% were females and 49.45% were males) with the diagnosis of extrapulmonary tuberculosis were obtained from 71 articles (11 articles published in international peer-reiew journals, 60 articles in national peer-review journals). Main results are shown in table. The most clinical symptoms were fever (41%), sweating (20%), weight loss (17%), fatigue (15%). 6% had contact with active tuberculosis patients.
Conclusion: Tuberculosis is among the most common infections in Turkey. Diagnosis of pulmonary and extrapulmonary diagnosis is challenging. Extrapulmonary tuberculosis should be taken into consideration in the differantial diagnosis of patients admitting with symptoms such as night sweating, cough and fever. Evaluation of the clinical findings and good microbiologic sampling will increase the proper diagnosis of the cases.

P1564 Tuberculosis management and drug resistance in Hlabisa sub-district, KwaZulu Natal

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Background: Hlabisa sub-district, in rural KwaZulu Natal, has a population of 220,000 and is served by a 300 bed hospital linked to 15 fixed primary healthcare (PHC) clinics. HIV sero-prevalence is 40%. 3000 tuberculosis (TB) cases are notified annually. TB accounts for 33% of in-patient mortality. A review of local TB control was undertaken to identify areas of public health concern.
Methods: South African National Tuberculosis Control Guidelines (2004) were followed. Diagnosis was made on sputum microscopy, clinical features and X-Ray. New patients received standard six month therapy using rifampicin, isoniazid, pyrazinamide and ethambutol. Retreatment cases and defaulters received eight months of treatment with a different protocol including intramuscular streptomycin for the first 5 months. Community treatment outcomes from October 2004-April 2005 were recorded. 337 TB in-patients were interviewed in 2005. HIV testing was offered and details of prior TB therapy recorded. Sputum was cultured on all hospital and community retreatment cases, defaulters and smear positive patients who failed to become smear negative on standard therapy.
Results: Sputum smear positivity in TB diagnosis was low (40%). Only 37% of smear positive patients were cured. 29% were lost to follow-up before the end of therapy. Defaulting was attributed to financial/transport difficulties, lack of community treatment supporters and inadequate tracing resources. 70% of TB in-patients were HIV positive. 104 (38%) had received previous TB treatment and 28 (8%) had been treated multiple times. One or more courses of prior TB therapy was associated with an increased risk of multi-drug resistant tuberculosis (Relative Risk: 1.86 [95% Confidence Interval: 1.25–2.70]). 60% of all hospital and community patients with rifampicin/isoniazid drug resistance were also resistant to streptomycin. 2 patients had Extremely Drug Resistant (XDR) TB.
Conclusions:
1. TB & HIV overwhelms district hospitals in KwaZulu Natal
2. Too many patients are lost to follow-up
3. Previous TB treatment is associated with the development of drug resistance
4. Streptomycin may no longer be an effective second line drug in this setting

P1565 Increasing incidence of extrapulmonary tuberculosis but stable rates of resistance of *Mycobacterium tuberculosis* isolates to first-line anti-tubercular drugs in Kuwait, 1996–2005

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Objective: Continuous nationwide monitoring of drug-resistance levels is important for proper control/management of tuberculosis (TB). The aim of this study was to determine the incidence of pulmonary and extrapulmonary TB and trends in drug resistance patterns among all

Mycobacterium tuberculosis strains isolated over a ten-year period in Kuwait.

Methods: Drug susceptibility data for *M. tuberculosis* isolates recovered from all pulmonary and extrapulmonary TB patients in Kuwait from January 1996 to December 2005 were collected and analysed. Patients were divided into Kuwaiti nationals and expatriates. Prior treatment status was not recorded.

Results: From 1996 to 2005, 5399 nonrepetitive culture-positive TB cases (56% from pulmonary sites and 44% from extrapulmonary sites) among 917 (17%) Kuwaiti nationals and 4482 (83%) expatriates were identified. Although the total number of TB cases remained nearly same, downward and upward trends in the rate of *M. tuberculosis* isolates recovered from pulmonary and extrapulmonary specimens, respectively, were observed. Overall resistance rates were as follows: any drug, 12.5%; isoniazid, 9.1%; rifampicin, 1.1%; ethambutol, 2.0%; streptomycin, 4.3% and multidrug resistance (MDR), 0.9%. The resistance rates over the study period remained nearly same. However, significantly higher resistance rates for rifampicin and MDR among pulmonary versus extrapulmonary cases and for any drug, isoniazid and ethambutol among isolates recovered from expatriate versus Kuwaiti patients were observed.

Conclusions: The total number of active TB cases from 1996 to 2005 remained nearly same in Kuwait. Moderate and stable single drug resistance (<10%) and low MDR rates (<1%) were found among *M. tuberculosis* strains. The increasing incidence of extrapulmonary TB cases suggests that majority of active TB cases in Kuwait are occurring due to reactivation of previously acquired infection. The findings also highlight the importance of ongoing control measures to limit the development and spreading of drug-resistant *M. tuberculosis* strains in Kuwait.

Supported in part by KURA grant MI 02/04.

P1566 Treatment outcomes of tuberculosis in HIV-infected patients after initiation of anti-retroviral therapy in a resource-limited setting

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Objectives: To study the clinical outcome of tuberculosis (TB) treatment after initiation of ART in a resource-limited setting with high TB burden.

Methods: A retrospective cohort study was conducted in co-infected HIV and TB patients who had received anti-TB regimen and were subsequently initiated ART between January 2004 and December 2005. TB was diagnosed by clinical features, positive acid fast stain, and/or positive culture. Outcome of TB treatment was assessed after 48 weeks of ART.

Results: There were 188 patients with a mean (±SD) age of 36 (± 8) years; 68% were males. The mean (±SD) body weight was 53.3 ± 8.5 kgs. Median (IQR) CD4 cell count and plasma HIV-1 RNA at the time of TB diagnosis were 36 (15–77) cells/mm^3 and 5.6 (5.2–5.9) log copies/mL, respectively. Of all, 111 (59.0%) patients received nevirapine-based ART and the others received efavirenz-based ART. Median (IQR) duration from TB treatment to ART initiation was 1.7 (1.2–3.3) months. At 48 weeks, 144 (76.6%), 21 (11.2%), 11 (5.8%), 7 (3.7%) and 5 (2.7%) patients were cure/completed treatment, lost to follow-up, transferred care, died and receiving TB treatment (recurrent or drug resistant). Four of 7 (57.1%) causes of death were related to TB and one of these was related to TB immune reconstitution inflammatory syndrome (IRIS). After 48 weeks of ART, median (IQR) CD4 cell count was 232 (166–347) cells/mm^3 and mean (±SD) body weight was 61.1 (±11.1) kgs; both were significantly different when compared to baseline values ($P < 0.05$). By multivariate analysis, patients who received efavirenz-based ART had a higher tendency of cure/completed treatment of TB than those who received nevirapine-based ART after adjusting for age, baseline CD4 cell, baseline body weight, site of TB and timing from TB diagnosis to ART initiation ($P = 0.057$, OR=2.179, 95% CI=0.977–4.878).

Conclusions: Treatment outcome of TB among co-infected HIV and TB patients who subsequently receive ART is favorable. Recurrent or drug resistance rate is less than 3%. The further collaboration between HIV and TB program will lead to both successful scaling up of ART and effective treatment of TB among co-infected HIV and TB patients in the resource-limited settings.

P1567 Study of Chernobyl catastrophe influence on bovine TB epizootic process intensity in radioactive polluted and "clean" territories of Ukraine in 1983–2007

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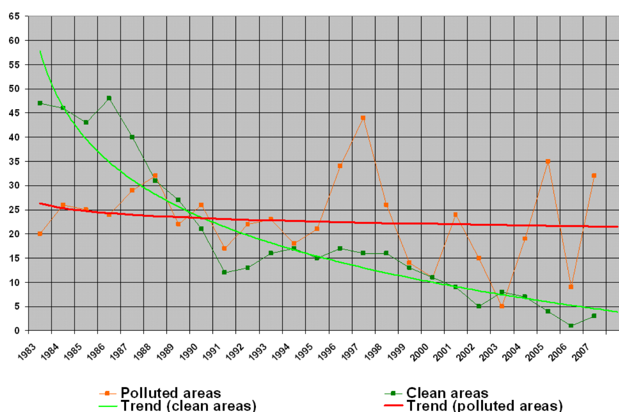
Objectives: As a consequence of Chernobyl catastrophe more than 144 000 ha of the agricultural lands were polluted by radiation. The considerable amount of cattle, including those reacted to tuberculin, is kept there. The aim of this work was to study the epizootic intensity of bovine TB in radioactive polluted territories of Ukraine before and after Chernobyl catastrophe as well as to compare it with such a situation in the territories free from radiation.

Methods: The most polluted are Kyiv, Rivne, Zhytomyr, Cherkasy and Chernihiv regions ("polluted" areas). Other regions are relatively "clean". The epizootic situation intensity was assessed with the help of such indices: incidence (on 100 000 heads) and focal index. Incidence was calculated as ratio of the amount of newly detected cattle reacted to tuberculin to the total amount of cattle in the period of 1 year. Focal index was calculated as ratio of the amount of newly detected cattle reacted to tuberculin to the amount of outbreaks in the period of 1 year.

Results: The graphic shows that before the catastrophe in 1986 the incidence in "polluted" areas was 20–25, in "clean" – 47–43. From 1987 till 2007 epizootic situation in "clean" areas was characterised by steady decreasing of the incidence from 40 to 3 respectively. In "polluted" areas the incidence had the tendency to increase slightly from 24 in 1986 to 32 in 1988 and thereafter it is decreasing till present time, however in the same time it exceeds the level typical for "clean" areas. Such a situation is well retraced with the help of trend lines – the trend line of "clean" territories is decreasing much more steeply then the trend line of "polluted" areas which is decreasing very gradual.

The epizootic process intensity is similarly characterised by the focal index. In 1983–85 this index in "clean" areas exceeded such in "polluted" upon the average in 1.61 times, in 1990–92 this ratio was in the range of 1. In 2006–07 the average amount of infected cattle per 1 outbreak in "polluted" areas exceeded the same index in "clean" areas in 2.72 and 1.96 times respectively.

Conclusion: Obtained data demonstrates more intensive epizootic process of bovine TB in radioactive polluted areas. This could be explained by the influence of high radiation doses onto animal organism as well as onto the biological properties of mycobacteria.



P1568 Evaluation of the sporicidal and tuberculocidal activity of the "high level disinfectant" lauryl dimethyl benzyl ammonium bromide

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Objective. To evaluate the sporicidal and tuberculocidal activity of two lauryl dimethyl benzyl ammonium bromide formulations (LDBAB) commercialised in Venezuela as high level disinfectants.
Methods. Sporicidal activity was evaluated with the use of membrane filters against a challenge inoculum of 106 *Bacillus atrophaeus* spores. Tuberculocidal activity was evaluated against *Mycobacterium tuberculosis* H37Rv using the quantitative suspension test described in the European Standard EN14348:2005.
Results. When tested at the concentrations indicated on their respective labels, both LDBAB solutions failed to show either sporicidal or tuberculocidal efficacy.
Conclusion. The LDBAB products tested are widely used in Venezuela for the disinfection of surgical and dental instruments. Public health authorities, as well as medical and dental professionals must correctly identify LDBAB as a low level disinfectant and discourage its use for instrument disinfection, as this practice may cause iatrogenic infections.

P1569 Effect of intensive education on compliance of tuberculosis patients in low socio-economic status in Bangladesh

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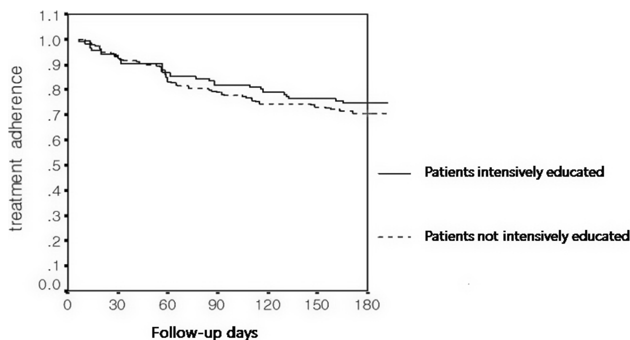
Objectives: Directly observed therapy short-course (DOTS) is main strategy facing TB in endemic area. However, Bangladesh is still one of eight countries in which new cases were not detected by DOTS program. We treated tuberculosis patients who were functionally out of reach of DOTS in Bangladesh-Korea Friendship Hospital in Bangladesh. Because compliance is the key of successful outcome in hospital-based treatment, we performed this study to evaluate whether education improves compliance of tuberculosis patients in low socio-economic status (SES) or not.
Methods: All patients who were diagnosed as tuberculosis from Jan 2005 to Mar 2007 were enrolled prospectively. We introduced intensive education strategy to the tuberculosis patients from May 2006. 6-month treatment complete rate in patients who were educated intensively were compared with the rate in control patients.
Results: During the period, 339 patients with tuberculosis visited the hospital. 26 patients who were transferred or stopped medication by physician's decision were excluded. 115 cases (37%) were educated intensively, while 198 (63%) weren't. Age (36.3 vs. 34.7, P = 0.37) and sex (male 58% vs. 64%, P = 0.40) were not different between the two groups. Treatment complete rate was 74.8% in intensively educated patients and 70.7% in control patients (P = 0.51).



Conclusion: Intensive education had no significant effect on compliance of tuberculosis patients in low SES in Bangladesh. Effective functioning of DOTS system is required in this area.

Epidemiology of fungal infections

P1570 Fungiscope – first Romanian multicentre study on fungaemia: preliminary results

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Objectives: This paper presents the preliminary results of a multicentre study on fungaemia made under auspices of the Romanian Society of Medical Mycology and Mycotoxicology in four tertiary hospitals from Iasi and Cluj-Napoca (Romania) between 2002 and 2007. The aim of this study is to evaluate the species distribution of fungal strains isolated from bloodstream cultures.
Methods: The study has included a total number of 3896 blood cultures from patients hospitalised in departments of cardiovascular surgery, general surgery, intensive care and infectious diseases. The range of age varied between 12 days and 82 years. The major clinical sign which required the bloodstream cultures has been the persistent fever. The presence of an intravenous central catheter, invasive surgery and previous broad spectrum antibiotic therapy have been the most frequent risk factors. In order to detect the fungal strains we used the Hemoline Performance Duo bottles, the BacT/ALERT®FA bottles, and the BacT/ALERT Microbial Detection System. The isolated strains have been identified on the basis of morphological and biochemical features, using specific tests.
Results: The percentage of fungaemia was 1.54% (60 positive blood cultures). The frequency of fungal species implied in fungaemia aetiology was: *Candida albicans* 35%, *C. parapsilosis* 15%, *C. tropicalis* 5%, *C. pelliculosa* 5%, *C. krusei* 5%, *C. sake* 5%, *C. glabrata* 3.33%, *C. dubliniensis* 1.66%, *C. norvegensis* 1.66%, *C. norvegica* 1.66%, *C. kefyr* 1.66%, *C. intermedia* 1.66%, *C. valida* 1.66%, *C. famata* 1.66%, *C. guilliermondii* 1.66%, *Rhodotorula glutinis* 3.33%, *Cryptococcus neoformans* 1.66%, *Trichosporon asahii* 1.66%, *Acremonium* spp. 3.33%, *Pseudallescheria boydii* 1.66% and *Fusarium solani* 1.67%.
Conclusions: The rate of positive blood cultures emphasising fungaemia is still low comparatively with those exhibiting bacterial infections. The study underlines the diversity of fungal strains isolated from bloodstream cultures. It can be noted a relatively low frequency of *Candida albicans* strains and the emergence of fungaemia due to non-*albicans* species accordingly to worldwide trends. More studies are necessary in order to evaluate the antifungal susceptibility profile, especially in species with high risk for resistance to usual antifungal drugs.

P1571 Fungaemia in Slovakia: a prospective, national study

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Objectives: To analyse the incidence of fungaemia/candidaemia, the aetiology of bloodstream fungal infections and trends in epidemiology in Slovakia.
Methods: All bloodstream fungal isolates from participating hospitals (all regional hospitals including all teaching hospitals) were collected and sent to central study laboratory for identification and sensitivity testing during the study period (September 2005 – March 2007). Germ-tube tests, chromogenic media (Chromagar, BBL), API 20C and YBC VITEK (bióMérieux) were used for identification of yeasts. The CLSI M44 method was used to test all yeasts isolates. Quality control strain *C. albicans* ATCC 90028 was required to be acceptable within 7 days of testing. Interpretive breakpoints for fluconazole and voriconazole were based on zones that correlated with CLSI-S2. All tests results were read by electronic image-analysis and interpreted and recorded with Biomic Plate Reader System.
Results: 204 fungal isolates from blood were obtained and analysed. Repeated cultures from the same episode were not evaluated. In 6 cases 2 different fungal pathogens were identified simultaneously. The recent incidence of fungaemia and candidaemia in Slovakia is 2.57/100,000/year and 2.16/100,000/year, respectively. Non-candida

fungal pathogens include *Acremonium strictum* (n = 36) (associated with nosocomial outbreak in single hospital), Geotrichum spp.(n = 3), *Pichia* spp. (n = 3), *Trichosporon* spp. (n = 3). 171 candida strains were isolated (83% of all fungaemic isolates) during the study period. *C. albicans* was the causative pathogen in 65 cases (38%) followed by *C. parapsilosis* (49/28.6%), *C. glabrata* (29/17%), *C. tropicalis* (15/8.7%), *C. guilliermondii* (7/4%) and *C. krusei* (6/3.5%). None of the *C. albicans* isolates was fluconazole-resistant; 13.5% of non-*albicans* isolates were fluconazole-resistant and 7% were voriconazole-resistant.
Conclusion: The results of this national study show the increase of the incidence of candidaemia in Slovakia comparing previous national survey. Our data also confirm the shift to non-*albicans* candida aetiology with various sensitivity to azoles.

P1572 Fungaemia in the university hospital of Antwerp: species distribution and antifungal susceptibility of isolates

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Objectives: To evaluate the species distribution of *Candida* bloodstream infections during a 5 year period (jan 2002-dec 2006) in the University Hospital, Antwerpen (Belgium) and to assess the antifungal susceptibility.
Methods: A total of 104 fungal bloodstream infections were reviewed. Biochemical test were used for species identification, and the isolates refered to the reference laboratory for confirmation of the identification. Susceptibility to 8 antifungal agents (amphotericin, fluconazole, ketoconazole, itraconazole, voriconazole, posaconazole, caspofungin and 5-flucytosin) was determined using Sensititre Yeast One plates (Trek Diagnostic Systems).
Results: The most common species were *Candida albicans* (52), *Candida glabrata* (33), *Candida krusei* (6) and *Candida parapsilosis* (6). There was no significant shift in species distribution over these 5 years. *Candida tropicalis* (4), *Candida inconspicua* (1), *Candida famata* (1) and *Cryptococcus neoformans* (1) were less frequently isolated. Susceptibilities to fluconazole, itraconazole and voriconazole are presented in the table (R: Resistant; SDD: dose dependently susceptible; S: Susceptible). The fluconazole resistant *C. albicans* isolate was also resistant to itraconazole en voriconazole.
Susceptibility to 5-flucytosin was 100%. All *Candida* isolates had a MIC ⩽1 for caspofungin, while 99 isolates had a MIC ⩽1 for amphotericin, the remaining 5 isolates had a MIC=2 for amphotericin.

Species (n)	Fluconazole, n (%)			Itraconazole, n (%)			Voriconazole, n (%)		
	R	SDD	S	R	SDD	S	R	SDD	S
C. albicans (52)	1 (2)		51 (98)	3 (58)	1 (2)	48 (92)	1 (2)		51 (98)
C. glabrata (33)	7 (21)	14 (42)	12 (36)	17 (52)	2 (6)	14 (42)	3 (9)	2 (6)	28 (85)
C. krusei (6)	5 (83)	1 (17)		1 (17)	5 (83)		1 (17)		5 (83)
C. parapsilosis (6)			6 (100)			6 (100)			6 (100)
C. tropicalis (4)			4 (100)		3 (75)	1 (25)			4 (100)
C. famata (1)		1			1				1
C. inconspicua (1)		1			1				1
C. neoformans (1)			1			1			
Total (%)	**12.5**	**16.3**	**71.2**	**20.2**	**12.5**	**67.3**	**4.8**	**1.92**	**92.3**

Conclusions: *C. albicans* and *C. glabrata* were the most prevalent species in bloodstream infections during 2002–2005. These findings are concordant with previously reported results. Fluconazole resistance in *C. albicans* was rare, supporting the use of fluconazole as the first choice treatment in patients with *C. albicans* fungaemia. Prevalence of fluconazole resistance in *C. glabrata* and *C. krusei* was 21% and 83%, respectively. The other non-*albicans Candida* spp. were susceptible or dose dependently susceptible to fluconazole, itraconazole and voriconazole.

P1573 Characteristics of fungaemias in western Malaga over a period of six years

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Objectives: The aim of this study was to investigated the clinical and epidemiological characteristics, as well as the sensitivity of fungaemia diagnosed in our hospital the last six years.
Methods: A retrospective study was conducted from patients with fungaemia from January 2001 to December 2006. The identification was carried out through the test filamentation and the automated system MicroScan RYI® (Dade Berhing). The susceptibility test was performed using microdilution system of Sensititre® (Izasa).
Results: We identified 105 episodes of fungaemia, 65.7% of them were male with an average age of 59.5 years. In 87.6% of cases, the patients had a primary disorder being neoplasias (42.9%), diabetes (31.4%), hypertension (24.8%) and chronic obstructive pulmonary disease (24.8%) the most frequent. The septic shock (39%) and fever (28.6%) were the most frequent clinical manifestations. The catheter (20%) was the most common origin of fungaemia. The risk factors were the invasive procedures (75.2%) and treatment antibiotics before isolation (91.4%). *C. albicans* was the specie more isolated (46.2%), followed by *C. tropicalis* (19.2%), *C. parapsilosis* (15.4%), *C. glabrata* (7.7%), *C. krusei* (5.8%) and others (5.7%). The susceptibility is shown in Table 1. All strains of *C. parapsilosis*, *C. lusitaniae* and *C. albicans* were susceptible to all antifungals agents tested.
The mortality of fungaemia was 52.4% being more frequent in the first 20 days. Only 20% of patients received antifungal agents before isolation, so the treatment was changed in 75.2% of cases after the report microbiology.

Table 1. Percentage of antifungal susceptibility in invasive yeast

Antifungal	Susceptible	S-DD	Intermediate	Resistant
Amphotericine	100%	–	–	–
Ketoconazole	94%	–	5%	1%
5-Fluorocytosine	95%	–	5%	–
Itraconazole	84.2%	12.9%	*	3%
Fluconazole	88.1%	8.9%	**	3%
Voriconazole	100%	–	–	–

*S-DD: Susceptible-dose dependent.

Conclusions: Fungaemia by *C. albicans* was the most frequent, but more than 50% of these were due to "non-*albicans*". *C. glabrata*, *C. tropicalis* and *C. krusei* were less susceptible species, with an overall resistance to fluconazole from 3%. Mortality in our environment was very high, highlighting the importance of microbiological report to appropriate treatment.

P1574 Nosocomial fungaemias at a general hospital: the change of epidemiological and clinical characteristics. A comparative study of 2 cohorts (1993–8 vs. 2002–05)

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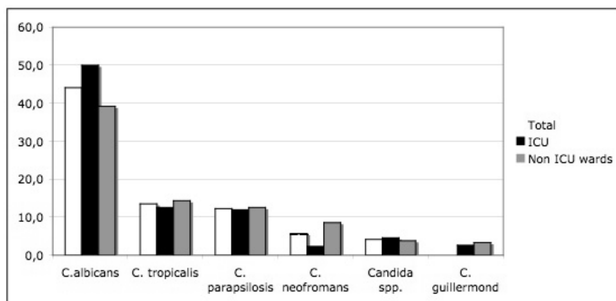
Objectives: nosocomial fungaemias (NF) have a high mortality rate; in the last years its incidence has increased. The aim of this study was to evaluate epidemiological and clinical aspects of 2 different cohorts of patients with NF and their changes between 2 different periods.
Patients and Methods: observational and comparative study of 2 cohorts of non-paediatric patients with NF admitted at a university affiliated hospital. Data collection from clinical records has been done according to a standard protocol. We analysed epidemiological, clinical, microbiological and laboratory data and changes in the 2 cohorts; the first one correspond to our historical cohort studied in 1993–1998 (P1)

and the second one to the period from 2002 through 2005 (P2). Patients with NF were identified by review of results of blood cultures from the hospital microbiology laboratory. Fungal isolation, identification and sensitivity test (Biomerieux) were performed by standard criteria. Mortality was assessed till 30 days after diagnosis of NF.
Results: 81 patients were studied during P1 and 107 during P2; incidence was 9/10000 in P1 and 15.8/10000 admitted-patients in P2 ($p < 0.05$). Mean age was 52 years in P1 and 61 years in P2 ($p < 0.05$); 90% in P1 and 67% in P2 were patients admitted in Surgical Wards ($p < 0.05$). In P1 65.4% NF were due to *Candida albicans* but in P2 only 44.8% ($p < 0.05$); 41% patients in P1 and 19.7% in P2 had also bacteraemia; diabetes was present in 12.3% in P1 and in 25.2% in P2 ($p < 0.05$). All of the patients had previously received at least one course of broad spectrum antibiotics. A statistically significant difference ($p < 0.05$) in predisposing conditions was identified in: central intravenous line rate (100% in P1 and 73% in P2), previous surgery (43.2% in P1 and 78.5% in P2). Acute severity of illness at onset and complications (respiratory distress, renal failure and intravascular disseminated coagulation) were more frequent in P2 ($p < 0.05$). Mortality rate was similar in P1 and P2 (50.4% and 50.1%, respectively).
Conclusions: frequency of NF has increased and *Candida* non-*albicans* is now more frequent than *C. albicans*. The association of bacteraemia is now higher. NF is associated with a high mortality rate but although acute severity of illness at onset and complications are now more often, mortality remains the same.

P1575 Fungal bloodstream infections in a citywide network in Bogotá, Colombia, 2001–2006

J.A. Cortes, A.L. Leal, G. Buitrago, J.S. Castillo on behalf of GREBO

Background: Fungal infections are common among patients in the icu, those with cancer and other forms of immunosupression. Bloodstream fungal infection are specially important due to the high mortality. Sentry information about Colombia is limited and do not reflect the real epidemiology.
Methods: A citywide network with 23 public and private tertiary hospitals was established. Laboratory information was transferred monthly to a central data base and compiled by the use of Whonet software (WHO, ver 5.4). Bloodstream infections were selected and those with a fungal isolate were analysed. Infections were stratified according to the place of acquisition (intensive care unit – ICU, non ICU wards).
Results: Between 2001 and 2006, a total of 35016 bloodstream infections were detected. Of those, 3.9% were fungal isolates (1366). *C. albicans* was the most commonly microorganism identified. Non identified species of *Candida* represented 4.2% of the isolates and decresed over time.



The figure shows the proportion of fungal isolates among patients in the icu and in non-icu wards. Of note, *Cryptococcus neoformans* was the fourth more common isolate among patients with fungaemia out of the ICU.
Discusion: *C. albicans*, followed by *C. tropicalis* and *C. parapsilosis* were the most frequently found aetiology of fungaemia. This pattern is different to that found in other parts of the world. A high frequency of HIV + patients in third level hospitals are responsible for the high frequency of *Cryptococcus* isolates. *C. glabrata*, a *Candida* species easily found in USA and Europe is scarce in our country.

P1576 Factors associated with mortality in patients with fungaemia

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Objective: A semi-national surveillance program of fungaemia in Denmark has revealed a notably high incidence. Therefore we have investigated the mortality, underlying conditions and host factors in fungaemic patients in 2006.
Methods: Participants are 6 departments of clinical microbiology serving the greater Copenhagen area and major parts of Zealand, Funen and Jutland (population 2,667,021). From each episode of fungaemia, a specimen is sent to the National Reference Laboratory for verification of species identification and susceptibility testing (EUCAST discussion document 7.1). A pro forma was provided to gather information on the patient, concomitant infections, treatment, procedures, and 30-day mortality, and on the time course of the mycological diagnosis. Fisher's exact test was used for comparison of mortality rates.
Results: A total of 316 episodes of fungaemia were recorded corresponding to a rate of 12/100,000 population. *C. albicans* encountered for 53% of the cases, *C. glabrata* for 21%, *C. krusei* for 8%, *C. parapsilosis* for 6% and *C. tropicalis* for 5%. 51% of the patients were at the ICU at the time of fungaemia, 56% had undergone surgery, which in most cases was abdominal (81%), while only 5% were leucopenic (<0.5 mia/L).
Overall 30 day-mortality was 38%, but 29% (81/280) among patients who received antifungal treatment. The mortality was higher in ICU patients, in the patients on mechanical ventilation (both 47%, P 0.0001) and in patients with leukocyte count of >10 mia/L (43%, P 0.0086). Mortality was not dependent on use of steroids, parenteral nutrition, dialysis, prior surgery or presence of underlying haematological disease. Species dependent mortality was *C. krusei* 36%, *C. tropicalis* 33%, *C. glabrata* 32%, *C. albicans* 28% and *C. parapsilosis* 25%, and a trend towards a higher mortality in the patients with *C. glabrata* fungaemia receiving fluconazole vs. caspofungin as first antifungal compound was observed (48% (12/25) vs. 17% (3/18) P 0.0521).
Conclusion: The incidence rate of fungaemia in Denmark is 3 times higher than reported in other Nordic countries and associated especially with ICU patients and patients with prior abdominal surgery. Mortality is highest in the sickest population, is associated with species and choice of initial antifungal treatment.

P1577 Invasive aspergillosis in haematological patients by exposure to laminar air flow: description of 65 cases between 2004 and 2006

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Objectives: Invasive aspergillosis (IA) occurred mainly in immunosuppressed patients (pts) with intensive chemotherapy and/or auto-or allogeneic hematopoietic stem cell transplantation. The objective was to describe IA and related care for pts hospitalised (+) or not (–) in a room with laminar air flow (LAF).
Methods: Proven and probable cases of IA (EORTC criteria) were collected prospectively between 01/01/2004 and 31/12/2006 in a university hospital and validated monthly by a multidisciplinary team. The demographic characteristics, the risk factors, clinical and mycological features and CT-scan reports were analysed.
Results: 65 IA were identified with a median age of 56 years.
Conclusion: Delay of onset between admission and diagnosis is similar between pts hospitalised in LAF rooms or not. The hypothesis on a fungal carriage before admission can be discussed considering environmental controls of *Aspergillus* contamination performed quarterly for each LAF room were satisfactory. The care management (CT-scans and mycological sample) was similar between the 2 groups of pts.

Pt characteristics	LAF– n = 34 (%)	LAF+ n = 31 (%)	P-value
Hemopathies			
Acute myeloid leukaemia (AML), secondary AML	28 (82)	24 (77)	0.66
Acute lymphoid leukaemia	2 (6)	4 (13)	
Other	4 (12)	3 (9)	
Status			
Induction chemotherapy	12 (35)	19 (61)	0.04
Other	22 (65)	12 (39)	
Stem cell transplantation <1 year	4 (12)	2 (7)	0.67
Risk factors			
Neutropenia <0.5 g/L	29 (85)	29 (94)	0.43
Corticosteroids	10 (29)	3 (10)	0.06
Clinical features			
Fever	33 (97)	31 (100)	
Pulmonary symptoms	26 (77)	26 (84)	
CT-scan performed	32 (94)	31 (100)	
Halo sign	27 (84)	25 (81)	
Excavation	1 (3)	0 (0)	
Serum *Aspergillus* antigen positive among performed samples (>1 ng/mL in more than 2 samples)	22/34 (65)	19/31 (61)	
Mycological samples performed	22	34	
Broncho-alveolar lavage (BAL)	13 (59)	17 (50)	0.81
Biopsies	3 (14)	7 (21)	
Other fluids	6 (27)	10 (29)	
Mycological samples positive (direct examination and/or cultures and/or antigenaemia) among performed samples	18/22 (82)	20/34 (59)	0.08
BAL	10/13 (77)	9/17 (53)	0.26
Biopsies	2/3 (67)	7/7 (86)	0.3
Other fluids	6/6 (100)	5/10 (50)	0.09
EORTC criteria			
Proven	4 (12)	10 (32)	0.07
Probable	30 (88)	21 (68)	
AI localisation			
Pulmonary	30 (88)	27 (87)	1.0
Sinus	1 (3)	1 (3)	
Disseminated	3 (9)	3 (10)	
Death 1 month after IA	8 (24)	4 (13)	0.34
Death 3 months after IA	20 (59)	11 (35)	0.08
Median delays for clinical diagnosis (days)			
Admission–IA diagnosis	17	17	0.49
Admission–neutropenia	5	3	0.34
Neutropenia–AI diagnosis	10	14	0.08
Median delays for complementary investigations (days)			
Clinical suspicion of IA–abnormal CT-scan	1	1	0.78
Positive antigenaemia–abnormal CT-scan (*n = 30*)	4	3	0.91
Abnormal CT-scan–positive antigenaemia (*n = 10*)	3	5	0.37

P1578 Eleven-year postmortem analysis of filamentous fungal infections in intensive care unit patients in a tertiary care hospital

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Objectives: Mould infections are an important and increasing cause of morbidity and mortality in patients hospitalised on intensive care units (ICU).

Methods: To investigate the incidence, underlying diseases, and pathological features of mould infection in ICU patients in a tertiary care hospital, all protocols of autopsies performed during a 11-year period (1997–2007) were reviewed.

Results: This retrospective analysis yielded 50 cases of mould infection. There were 32 males and 18 females. Median age was 59 years (range 25–81). The median SAPS II score was 52 (range 8–117) and the median ICU stay was 10 days (1–53). 47 cases with invasive mould infections (IMI) and 3 patients with aspergilloma were diagnosed. The most frequent isolates were *Aspergillus* species (94%) followed by *Fusarium* species (2%), *Scedosporium apiospermum* (2%) and Zygomycetes (2%). Fungal aetiology was entertained clinically in only 25 (53%) of the patients who were all treated with mould-active antifungal agents. However, in 37 (79%) patients IMI was the primary cause of death. Infected sites were lung 34 (72%), disseminated IMI 12 (26%), sinus 1 (2%). The major underlying conditions were hemato-oncological tumors in 14 patients (30%), followed by 9 patients after solid organ transplantation (19%), 7 patients with prolonged ICU stays after surgery (15%), 7 patient with rheumatologic or immunological disorders receiving high dose corticosteroids (15%), 5 patients with sepsis or cardiogenic shock (11%), 2 with chronic lung disease (4%), 2 HIV patients (4%) and one patient with nonseminomatous germ cell tumour (2%). Sixteen (34%) patients were neutropenic. The number of neutropenic patients decreased significantly (p = 0.047) during the study period. The median duration of ICU stay was significantly longer (19.5 vs. 5 days; p = 0.012) in patient without hemato-oncological diseases.

Conclusion: This survey found IMI to be frequent in ICU patients with a wide range of underlying conditions. The high incidence of not clinically entertained IMI confirms the importance of autopsy as a tool for quality control in medical diagnostic and therapeutic activity.

P1579 Four-year survey of zygomycosis in the Swiss Posaconazole Named Patient Program

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Background: Zygomycosis mainly due to *Rhizopus* and *Mucor* spp. is an emerging invasive mould infection with high mortality. Response rates to antifungal therapy up to 40% have been reported. Epidemiological data for Switzerland are lacking.

Objectives: To describe the clinical characteristics of patients with zygomycosis identified among the cohort of 45 patients with invasive fungal infections treated with posaconazole in a named patient program in Switzerland between 2003 and 2007.

Methods: Data on clinical presentation, diagnosis, microbiology, and outcome of 45 patients were retrospectively collected by questionnaire.

Results: 18 patients were diagnosed with invasive zygomycosis (16 proven and 2 possible cases according to the EORTC-MSG criteria). Eleven of the proven cases were reported between January, 2006 and April, 2007, when the program became better known. The median age of patients was 53 years (3 to 67), 80% were male. Underlying conditions included haematological malignancies in 50% (90% of whom were neutropenic at the time of diagnosis), solid organ transplantation in 17%, non-haematological malignancies, diabetes, and trauma in 11% each. Infection sites were pulmonary in 37% of cases, skin and bone in 26%, rhinocerebral in 21%, and disseminated in 11%. *Absidia* spp. were isolated in 31% of cases, *Mucor* spp. and *Rhizopus* spp. both in 19%, *Rhizomucor* spp. in 12%, and *Cunninghamella* spp. in 6%. Eighty-three percent of patients were pretreated for a median of 33 days (0 to 180) with a median of 2 antifungals (0 to 5) before switching to posaconazole. Most commonly used first-line drugs were amphotericin B deoxycholate (61%), liposomal amphotericin B (50%), voriconazole (50%), and caspofungin (22%). Reasons for switching to posaconazole were failure (56%), intolerance to previous medication (17%), and i.v to oral switch (22%). In addition to antifungal treatment 61% of patients underwent surgery. Outcome data were available for 15 patients: a complete response to treatment was recorded in 60%, a partial response in 20%, and failure in 20%. Patients with complete response were treated with posaconazole for a median of 4 months (1.5 to 13), those with partial response for 1.5 months (1 to 3), and those with treatment failure for 3 days (3 to 4).

Conclusion: This Swiss survey supports that zygomycosis is emerging. *Absidia* spp. were the predominant pathogen. New treatment options may improve the outcome of this life-threatening disease.

P1580 Fungal infections in liver transplant recipients

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Objectives: The incidence of fungal infections ranges 7–42% in liver transplantations. Invasive fungal infections usually occur in three months following transplant surgery. In liver transplant recipients 62–91% of fungal infections are caused by *Candida*. Although most specialists recommend antifungal prophylaxis for liver transplant recipients, the frequency of fungal infections differs from centre to centre, geographic locations patients live and to the type of transplant surgery done. We aimed to find out fungal infection incidence in liver transplant patients in

DEU Hospital (Izmir/Turkey) in order to make a decision for antifungal prophylaxis.

Methods: Between January 2003 and December 2006, 150 cases of liver transplantation were performed at our centre. Case records were examined retrospectively for fungal infection risk factors. Re-exploration, rejection, retransplantation, drainage catheters, reintubation, prolonged broad-spectrum antibiotic use, massive blood transfusion, and CMV infection were accepted as independent risk factors. The patients were grouped as definite, possible and probable diagnosis

Results: In this study 64 of 150 patients were consulted with infectious disease and clinical microbiology specialists because of post-transplant infections and five patients were required antifungal treatment. In three patients fungal pathogens were isolated either in peritoneal fluid or blood culture. In one patient *Candida albicans* was isolated from urine sample. *C. albicans* was isolated from all specimens. Case 3 was reexplorated for seven times and massive blood transfusion was performed. Through the patients given antifungal therapy, only in one patient any fungal pathogen was isolated (see table for details). Even there was no microbiological proof, clinical response was determined with antifungal therapy. There was no *Aspergillus* infections detected.

Conclusion: As a result the incidence of fungal infection is 3.2% in liver transplant recipients at our centre. The use of prolonged and broad-spectrum antibiotics and re-exploration were common risk factors in all cases. When possible and probable fungal infections were analysed, the incidence of fungal infections in liver transplant patients was lower than expected. According to these findings antifungal prophylaxis after transplantation is not recommended for the recipients in Dokuz Eylul University Hospital.

	Case 1	Case 2	Case 3	Case 4	Case 5
Re exploration	+	+	+	+	+
Retransplantation	–	–	–	–	–
Rejection	–	+	–	–	–
Use of prolonged and broad spectrum antibiotics	+	+	+	+	+
Drainage catheters	+	–	–	+	+
Massive blood transfusion	–	–	+	–	–
CMV infection	–	–	–	–	–
Intubation	+	+	+	–	–
Fungal agent	*C. albicans*	*C. albicans*	*C. albicans* *C. glabrata* *C. tropicalis*	*C. albicans*	Not detected
Pathogen isolated	Peritoneal fluid culture	Blood culture	Peritoneal fluid Blood culture	Urine culture	–
Date of fungal pathogen determined	321	171	18	9	48
Bacterial pathogen isolated	*Enterococcus faecalis*	*Comannonas* spp.	*Acinetobacter* spp. ESBL(+) *Klebsiella pneumoniae*	*Acinetobacter* spp. MRSA ESBL(+) *E. coli*	*Acinetobacter* spp. *Enterococcus* spp.
Antibacterial treatment	Meropenem Ciprofloxacin Vancomycin	Pip-tazobactam Vancomycin Ciprofloxacin	Pip-tazobactam Cefoperazone sulbactam Netilmicin Vancomycin	Pip-tazobactam Ciprofloxacin Meropenem Teicoplanin Cefoperazone-sulbactam Netilmicin	Pip-tazobactam Meropenem Cefoperazone-sulbactam Vancomycin Amikacin
Total antibiotic treatment	26 days	15 days	66 days	54 days	60 days
Antifungal treatment	Fluconazole	Amphotericin B	Caspofungin	Fluconazole Amphotericin B	Fluconazole
Prognosis	Alive	Exitus	Alive	Exitus	Alive

P1581 Clinical and epidemiological features of recent imported dermatophytoses in Spain

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Objective: A changing pattern in dermatomycoses has been reported in Europe due to recent waves of immigrants from developing countries and a lesser extent increasing of native travellers to these areas. Data about dermatomycoses in these populations are useful to know the predominant species and clinical features to accomplish these infections. The aim of this study was to describe mycological, clinical and demographic characteristics in recent immigrants and travellers in our area.

Methods: A retrospective analyse was carried out in immigrant population from developing countries including adopted children, and native travellers between January 2000 to February 2007. Specimens were examined according to reference methods. Clinical and epidemiological data were also recorded.

Results: A total of 1344 specimens from superficial cutaneous lesions were examined. 271 yield positive results for fungal strains and in 154 of them dermatophytes were isolated corresponding to 146 patients, 103 adults and 43 children (under 14 years old). The majority of patients came from Subsaharian Africa (52%). Among the remain immigrants the origin was Asia (7.5%), Central and South America (6.9%) and Maghreb (1.4%). A substantial proportion of dermatomycoses were isolated from native travellers (28.1%). In 4.1% cases the nationality was unknown. These dermatophytoses were distributed in tinea corporis (72.7%), tinea capitis (15.6%) and tinea unguium (11.7%). The most common dermatophyte species isolated was *Trichophyton rubrum* (47.4%), followed by *Trichophyton soudanense* (17.5%), *Trichophyton violaceum* (13.6%), *Microsporum audouini* (7.1%), *Trichophyton mentagrophytes* (5.8%), *Microsporum canis* (4.6%), *Epidermophyton floccosum* (1.3%), *Trichophyton rubrum* var. *granular* (1.3%), *Trichophyton tonsurans* (0.7%), and *Trichophyton verrucosum* (0.7%). All tinea capitis were isolated in adopted children, most of them from Subsaharian Africa who carried antropophilic dermatophytes.

Conclusions: Antropophilic dermatophytes were frequent in immigrant population, specially in paediatric population from Subsaharian African countries. Of note, a remarkable proportion of native travelers were infected by dermatomycoses. Surveillance and proper treatment would be necessary in immigrant populations and travellers from developing countries to prevent spreading infections.

P1582 Two specific strains of *Histoplasma capsulatum* causing mucocutaneous manifestations of histoplasmosis: a frequent manifestation of histoplasmosis in Latin America

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Objectives: Skin lesions, uncommon in US cases (<10%), occur in 38% 85% of cases reported from Latin America. Although these differences may reflect reporting bias, delayed diagnosis, or differences in host immune response among different ethnic groups, they also could result from genetic differences changing the pathobiology of the organism. It is possible that genetic differences among strains of H. capsulatum may alter the pathogenesis and clinical manifestations of histoplasmosis.

Methods: We examined the clinical features of patients with mucocutaneous manifestations of histoplasmosis and performed genetic analysis based on nucleotide sequence variations in the internal transcribed spacer regions of rRNA genes of *H. capsulatum* isolates of patients. Two pairs of PCR primers were designed to develop to amplify the ITS regions of *H. capsulatum*, 5′- TACCCGGCCACCCTTGTCTA-3′ and 5′-AGCGGGTGGCAAAGCCC-3′. These primers were based on the ITS sequence of Ajellomyces capsulatus, the ascomycetous teleomorph form of *H. capsulatum*, deposited in the GenBank (accession number U18363). Eight patients attending a tertiary-care hospital in southern Brazil were enrolled into the study. All case patients had skin cultures growing *H. capsulatum* at the mycology laboratory.

Results: Six of eight (75%) patients were HIV-positive and presented involvement of multiples organs by *H. capsulatum*. Two HIV-negative patients did not present evidence of involvement of other organs besides mucosa and skin. ITS sequencing of the *H. capsulatum* isolates from the 8 patients revealed two distinct strains. The 2 distinct fragments (Hc1, Hc2) differed from each other at 7 positions in the ITS regions. They were identical to strains of *H. capsulatum* isolated in patients from Colombia and Argentina, but different from strains isolated in US. The *H. capsulatum* clinical strains Hc1 was isolated in 6 patients and Hc2 in 2 patients with mucocutaneous manifestations of histoplasmosis. Both Hc1 and Hc2 strains were isolated in HIV-infected and non HIV-infected patients.

Conclusions: Mucocutaneous manifestations of histoplasmosis, which are frequently seen in Brazilian patients were caused by 2 specific strains.

Those strains have been isolated in patients with these particular clinical features of histoplasmosis in Latin America. Our study suggests that unique pathogenic characteristics among the Latin America species of *H. capsulatum* might explain its increased dermatotropism.

P1583 Hypersensitivity pneumonitis due to Pseudozyma sp. in a liver-transplanted child

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Hypersensitivity pneumonitis (HP) in children is rare and mainly associated with exposure to antigens in home environments.
L, a 5-year-old child, liver-transplanted at 11 months (with long-term tacrolimus therapy), lives on an old farm in Switzerland. Diagnosis was made based on clinical history, bilateral ground-glass and micro-nodular opacities on CT-scan, probable high antigen exposure, BAL lymphocytosis and confirmed by endobronchial biopsy, revealing lymphocytic infiltrate and non-necrotising peribronchiolar granuloma.
Environmental sampling (home, stable, barn, n = 38) was performed to prove antigen exposure. Precipitins against 22 different antigens were investigated. Significant precipitins (5 arcs, electrosyneresis) were observed for only one antigen, extracted from a yeast identified as Pseudozyma sp. by rRNA gene sequencing and isolated from two samples of oat grain. One year later, following corticotherapy and antigen exposure reduction, HP symptoms had regressed.
Pseudozyma genera are closely related to Ustilago, phytopatogenic fungi. Because the plantpathogenic dikaryotic state of the latter cannot be cultivated in vitro, Ustilago were not used to detect precipitins. Precipitins against the Pseudozyma antigen were retrospectively assessed in five Farmer's lung disease (FLD) cases, 12 exposed and 18 non-exposed control subjects. Precipitins were significant only in 3 out 5 FLD cases. Pseudozyma antigens could be used to assess exposure to Ustilago in serological diagnosis of HP.
This HP case is unusual due to patient's age, immunosuppression (contrasting with hyper-immune response in HP pathogenesis) and previously undescribed aetiology of HP.

P1584 *Pneumocystis jirovecii* colonisation in young people with HIV infection

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Objectives: The incidence of *Pneumocystis* pneumonia (PcP) has declined in AIDS patients in developed countries with the use of specific chemoprophylaxis and highly active antiretroviral therapy (HAART), but still being the major cause of morbidity and mortality. The use of molecular techniques for diagnosis has revealed the existence of both: immunosuppressed and immunocompetent carriers of *Pneumocystis*. The colonisation in subjects with HIV infection is a risk for developing PcP. In our environment there is not information about this situation. The aim was to investigate the frequency of colonisation by *P. jirovecii* in young subjects with HIV infection and risk factors associated to this colonisation.
Patients and Methods: Twenty patients followed since childhoods were included and their clinical, immunological stage and viral load was analysed. To determine the presence of *P. jirovecii* sputum and/or oral wash samples was obtained in a follow-up visit. PCR techniques were assayed at two independent loci: The mitochondrial region (mt LSU rRNA) and DHPS gene (mutations are associated with sulphonamides resistance). These mutations were identified by restriction analysis of polymorphisms (RLFP).
Results: The mean age of cohort was 13.7 years, being 11 of them male. In 10% a previous PcP episode was diagnosed. The rate of colonisation by P Jirovecii at the moment of the study was 40% (8/20). None of these patients shows mutations at DHPS gene. Statistical analysis does not shown relationship between the presence and absence of colonisation and, CD4+, CD8+ levels, or viral load.
Conclusions: There is a high rate of colonisation by *Pneumocystis* in young HIV infected patients which is associated with a high potential risk of suffering a PcP, especially when the strains have mutations at DHPS gene or patients suffered severe immunosuppression.
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P1585 Pulmonary aspergillosis following *Mycobacterium kansasii* lung disease

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Objectives: The aim of the study is to evaluate the incidence, clinical features and outcome of *Aspergillus* lung disease (ALD) in patients (pts) with *Mycobacterium kansasii* pulmonary disease.
Methods: Retrospective review of 5 cases of ALD from a cohort of 111 HIV-negative pts with *M. kansasii* disease, treated in a teaching hospital, between 1990 and 2005. Diagnosis of ALD was based on radiological signs, respiratory sample cultures and serology.
Results: Five men with a mean age of 59 years were diagnosed of ALD (4.5%, incidence rate 1.08 patient-years, 95% CI 0.35–2.53). All patients were smokers, 3 had COPD, 2 malnutrition and 1 was taking corticosteroids. Mean time from diagnosis of *M. kansasii* disease to the development of ALD was 23 months. ALD developed during treatment for *M. kansasii* in 2 patients. All 5 presented cavitary lesions in relation to *M. kansasii* disease. Most important clinical manifestations were hemoptysis (3), dyspnea (2) and weight loss (2). CT showed mycetoma in 3 cases and pseudonodular infiltrates in the other 2. Diagnosis was established by culture of respiratory samples (4) and histological examination (1). *Aspergillus fumigatus* was identified in the 4 positive culture cases. Precipitins were positive in 2 of the 3 patients in whom they were performed. Antifungal therapy was based on itraconazole (4 courses), voriconazole (3) and amphotericin B (1) with a mean duration of 135 days. Embolisation and surgical resection because of recurrent hemoptysis was required in 2 cases. Two patients died due to postoperative complications and lung cancer respectively, and 3 were alive after a 45.8-month mean follow-up.
Conclusions: ALD is not an uncommon complication of *M. kansasii* pulmonary disease and implies a poor prognosis. Reappearance of respiratory symptoms in any patient being treated or formerly treated for *M. kansasii* disease should raise suspicion of ALD.

Antiviral vaccines

P1586 Influenza virus vaccination in kidney transplant patients: serum antibody response to different immunosuppressive drugs

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Objectives: Influenza vaccine response in kidney transplanted patients has shown conflicting results, which can be associated with different immunosuppressive regimens. We prospectively assessed the humoral immunity to the trivalent inactivated influenza vaccine in kidney transplant adult patients.
Methods: 75 renal transplanted patients with at least six months of transplantation received one dose of inactivated influenza vaccine 2004–2005. Anti-hemagglutinin antibody response against each strain was measured by hemagglutination inhibition test before and monthly up to 6 months after. The geometric mean titer (GMT) of each strain was calculated using the log-transformed values from all subjects; the GMT was taken as the antilog of the mean of the transformed value. Logistic regression model was used to assess the impact of different immunosuppressive drugs on the vaccine response rates.
Results: One month after vaccination, 57.4% of patients acquired protective titers of antibodies to at least one vaccine strain. The GMT of H1N1 and H3N2 strains increased from 2.75 and 2.44 to 13.54

(P = 0.001) and 7.30 (P < 0.001) respectively. Pre and post-vaccination protection rates for H1N1 and H3N2 increased from 9.3% to 45.3% (P < 0.001) and 9.3% to 33.3% (P < 0.001). The H1N1 and H3N2 seroconversion rates after vaccination were 36% and 25.3%, respectively. There was no antibody response to influenza B virus. Compared to the use of azathioprine (AZA), Mycophenolate Mofetil (MMF) reduced the H1N1 [P = 0.011, OR 0.26 (IC95% 0.097–0.734)] and H3N2 [P = 0.033, OR 0.30 (IC95% 0.10–0.91)] protection rates and the seroconversion rates for the H3N2 strain [P = 0.036, OR 0.26 (IC95% 0.07–0.91)].
Conclusions: Renal transplanted patients submitted to an anti-influenza vaccination responded with antibody production against strains H1N1 and H3N2 of influenza A virus but not to influenza B virus. Use of MMF decreased the humoral immune response to the anti-influenza vaccine.

P1587 Antibody response to influenza immunisation in kidney transplant recipients receiving either azathioprine or mycophenolate: a controlled trial

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Objectives: This study aimed to assess the humoral immune response to influenza vaccination in adult kidney transplant recipients (KTRs) subjected to two immunosuppressive regimens containing either mycophenolate mofetil (MMF) or azathioprine (Aza).
Methods: Forty eligible KTRs (24 treated with Aza [KTRs-Aza] and 16 treated with MMF [KTRs-MMF]) and 40 matched healthy controls (HCs) were administered the trivalent 2006–2007 anti-influenza vaccine. Antibody (Ab) titers were measured before (pre-vacc) and 1 month after (post-vacc) vaccination. The proportion of protective Ab titers (i.e. ⩽1:40), the serological response (i.e. ⩽4-fold rise in titers) rates, and the magnitudes of change in titers were evaluated.
Results: KTRs and HCs were similar in serologic responses, magnitudes of change in Ab titers, and proportions of newly developed protective titers against all antigens. Whereas KTRs-MMF and KTRs-Aza were identical in magnitude of rise in titers as well as in serologic responses, KTRs-MMF did poorer in newly developing post-vacc protective titers against A/H1N1 (P < 0.05). The function of the transplanted kidney has not deteriorated after vaccination.
Conclusions: Anti-influenza vaccination was safe in KTRs and evoked Ab responses comparable to those of HCs. KTRs-MMF and KTRs-Aza responded almost equally to the vaccine. Annual anti-influenza vaccination can be recommended to all stable KTRs.

P1588 Effectiveness of the influenza vaccine – Problems with selection bias

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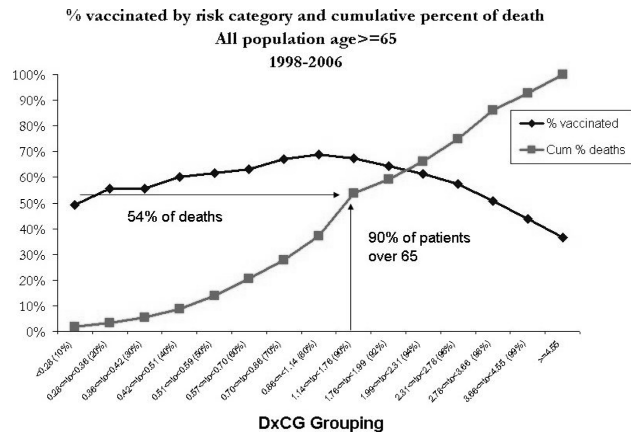
Background: Despite a lack of consistent evidence showing a large benefit, the influenza vaccine has been recommended for seniors in the US for almost 50 years. Only one randomised, controlled study showed a benefit in the elderly, and this diminished with age over 70. Placebo-controlled trials are unlikely to occur in the near future. Many observational studies have shown a benefit much greater than is plausible, considering immune senescence and the known impact of influenza on mortality. These observations have led some to believe that the benefits of the vaccine are truly unknown, and that perceived effectiveness may be due to wishful thinking and bias in the observational studies. We looked at vaccine uptake and underlying morbidity in our population to see if we could find insights into the potential bias of observational studies..
Kaiser Permanente is a medical care organisation in Northern California with almost 3.3 million members. All medical information is computerised, including diagnoses, vaccinations, and medications. The entire population is categorised into risk groups using DxCG® software (based on 140 diagnostic groups)
Methods: We studied Kaiser members over 65 years of age, during the years 1997–2004. We compared all-cause mortality in members vaccinated with any type of influenza vaccine with the unvaccinated.



Results: Mortality in the vaccinated is extremely low just after vaccination, then rises to more closely approximate the non-vaccinated at the end of a year. Mortality in the unvaccinated is very high right after vaccination, then diminishes over the year. These effects are independent of the influenza season, and reveal significant bias in observational studies of vaccinated vs. unvaccinated individuals.
Using DxCG ratings as a marker of underlying morbidity We also found that healthier people over 65 are more likely to get a flu vaccine, and that the likelihood of vaccination increases with underlying morbidity, up to a point. When underlying morbidity reaches a threshold (the sickest 10%), vaccination decreases with increasing morbidity.

P1589 Administration of DNA and protein vaccines containing gD genes of HSV1 and 2

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Objectives: Herpes simplex virus type 1&2 infect mucocutaneously and causes oralabial and genital infection in humans, followed by the establishment of a latent infection in the sensory ganglia and can reactivate for life. The prevalence are increasing worldwide and varies widely with generally higher rate in developing than developed countries. A need for effective vaccine remains the preferred strategy for control of HSV infections.
Methods: The PCR amplified HSV gDs were cloned into desired plasmids in order to construct DNA vaccine or recombinant expression vector. Confirmed baculovirus-based recombinant plasmids were transfected into Sf9 cells, cultured in serum-free Grace's medium at 27°C and monitored daily for observation of cytopathic effects. After 72 hours, the recombinant baculoviruses were harvested from the cell culture medium. The viral stock was amplified by re-infecting insect cells and titrated as pfu. The Sf9 cells were infected with gDs-baculovirus and incubated at 27°C for various times to express the recombinant protein of interest.
Results: The constructs were confirmed by colony PCR, Restriction Enzyme Analysis and sequencing. The protein productions in Cos-7 and insect cells were shown by western blot analysis. The recombinant plasmids and protein of interest were injected in different groups of BALB/c mice. The expression vector without any fragment and Sf9 cells were used as negative control and injected to control groups. The inductions of humoral and cellular responses were evaluated.
Conclusion: Although vaccination of mice with a plasmid expressing the neutralising antigens induced humoral and cellular immune responses, but the antibody titer were significantly lower than that of antibodies induced by the subunit protein vaccines. Furthermore, the plasmid DNA as well as recombinant protein vaccine could protect the mice against HSV-1 and HSV-2 lethal challenge.

P1590 Viability of live-attenuated vaccine strains in a pharmaceutical dump

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Objectives: To assess the viability of discarded and buried vaccine strains, we examined vaccines that had been buried for >20 years in the 12,000m^2 waste dump of the bankrupted Istituto Sieroterapico Milanese (ISM) in Milan, Italy. This dump area was recently reclaimed after nearly a decade of abandonment. Some recovered material, including vaccines against human and animal diseases such as rabies, poliomyelitis, anthrax, Newcastle Disease (ND) and Canine Distemper (CD), were recovered in hermetically sealed vials, so their contents might have been totally or partially preserved.
Methods: We selected and analysed two lyophilised live attenuated CD Virus (CDV) vaccines, recovered and unearthed in the dump.
An amplification of a specific 550bp fragment of CDV NP gene was performed by nested RT-PCR. The PCR-positive specimens were then submitted to the viability tests through the inoculation of the resuspended vaccine on a confluent monolayer of permissive cell cultures (Vero, African green monkey kidney) and the detection of the specific cytopathic effect. As positive control, we used a vaccine against the CDV currently in use, Caniffa-CHL, (Merial Italy S.p.a., Milan, titre $\geqslant 10^3$ CCID_50).
Results: CDV lyophilised vaccine samples resulted positive to the PCR-analysis, attesting the competence and integrity of the viral genome in the vials.
Three days after the inoculation of the sample on Vero cells we observed the specific cytopathic effect (lysis plaque formation).
Conclusions: This result confirmed that CDVs recovered in the dump were endowed with viability, thus showing they retained their replicative ability, as previously reported for ND Virus vaccine strains (Amendola et al, Emerg Infect Dis. 2007). The discovery of the maintenance of the replication activities of lyophilised vaccine strains after 30 years of burial is relevant because it emphasises that such preparation may preserve viable strains. This event seems occur independently of the virus species, and therefore it is also possible that viruses that can cause hazard to humans (as poxvirus or poliovirus) may persist for years and be dangerous at the moment of manipulation while reclaiming activities take place. Altogether, these evidences strongly underline the need of guidelines for the disposal of such particular products, such as vaccines and their by-products, and the lack of studies aimed at evaluating the environmental infection risk from the uncontrolled burial of industrial bio-material.

P1591 Effect of vaccination for haemorrhagic fever with renal syndrome: analysis of 5-year experience

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Objectives: Haemorrhagic fever with renal syndrome (HFRS) is endemic pyrogenic disease caused by Hantaan virus, and characterised by fever, renal failure, and bleeding. In Korea, hundreds of patients are newly diagnosed as HFRS for recent years. We evaluate the effect of HRFS vaccination (Hantavax, inactivated Hantaan virus solution [ROK 84–105] 4,096 ELISA unit/mL, Green Cross vaccine, Korea) on clinical features from our experience for years.
Methods: A total of 214 patient were included from a tertiary Korean hospital. One hundred and thirteen patients were positive in Hantaan virus antibodies with passive haemagglutination methods or immunochromatography between 2002 and 2006, and 19 of these were diagnosed as HFRS on the basis of clinical manifestations (classified as Group 1). The other 101 patients (Group 2) were treated as HFRS between 1986 and 1990 when HFRS vaccine was not developed. Vaccination were confirmed by patient's chart review or telephone surveys.
Results: The patients' ages (mean ±SD) were 44.0±23.1 vs. 45.4±14.1, hospitalisation days (mean ±SD) 13.6±8.2 vs. 10.5±9, renal failure was developed in 12 (63.2%) vs. 96 (95.0%) (P < 0.05), flank pains 2 (10.5%) vs 59 (58.4%) (P < 0.05), haemodialysis was required in 0 (0.0%) vs. 17 (16.8%), mortality 1 (5.3%) vs 12 (11.9%) in group 1 vs. group 2, respectively. There was significantly low incidence (5%, 3/56) of HFRS in vaccinated people compared with nonvaccinated (21.5%, 11/51) (P < 0.05).
Conclusion: We concluded that vaccination has contributed to declined incidence and decreased severity of HFRS.

P1592 Long-term follow-up of new-borns of HBsAg positive mothers vaccinated against hepatitis B

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Objectives: Protection after combine passive-active immunisation against hepatitis B was investigated in 685 neonates of HBsAg positive mothers in north-eastern part of the Czech Republic.
Methods: Combine passive-active immunisation was commenced in 1988 year and the number of immunised children gradually increased till the end of 2006 year. The children received hepatitis B immunoglobulin at birth and three 10 μg doses of plasma-derived or recombinant vaccine at interval 0, 1 and 6 months (30 children of HBeAg positive mothers at interval of 0, 1 and 2 months). Blood samples were obtained after immunisation, at 2 years of age, and biennially thereafter. Samples were tested by ELISA for HBsAg, anti-HBs and anti-HBc. The immunisation schedules were completed in 657 children.
Results: Anti-HBs antibodies were tested in 641 children after immunisation; protective anti-HBs antibodies were proved in 598 of them (93%). Twenty-six children without anti-HBs after immunisation received one identical booster vaccine; protective anti-HBs were proved in 24 of them. Vanishing of protective anti-HBs antibodies was detected in 30%, 49% and 62% children in 5, 10 and 13 years of their life. Vertical transmission of hepatitis B virus with chronic HBsAg carrier status was proved in 2 children, one of them was infected by escape mutant of HBsAg. Asymptomatic infection with long-term presence of anti-HBc antibodies was observed in 9 children. The anamnestic response with double increase of anti-HBs without revaccination was proved in 63 children.
Conclusions: The vaccination of neonates of HBsAg positive mothers against hepatitis B was successful and practically interrupted the vertical transmission of hepatitis B virus.
Grant support: IGA Ministry of Health, Czech Republic, NR/9257-3/2007.

P1593 Prevalence for serological markers of hepatotropic viruses A, B, and C, and hepatitis A and B vaccination needs in a prospective cohort of patients with chronic liver diseases awaiting liver transplantation

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Vaccination against hepatitis A and B is widely recommended in patients with chronic liver disease, especially those awaiting a liver transplant, because of the increased risk of developing a life-threatening hepatitis (HAV and HBV), or HVB reactivation when transplanted.
Objectives: The objectives of this study were to investigate the prevalence of markers of hepatotropic viruses and to identify candidates for vaccination against hepatitis A and B in a prospective cohort of patients waiting for a liver transplant in a teaching hospital in Paris, France.
Methods: Between March 2006 and November 2007, 80 patients on the liver transplantation waiting list were studied for socio-demographic, epidemiological, clinical, routine biological data and serological markers for hepatitis viruses including HAV, HBV and HCV.
Results: The mean age was 51 years (19 to 66). M/F ratio was 2.5. Of these 80 patients, 96% had cirrhosis, mostly due to alcohol abuse (37.5%), chronic viral hepatitis (31%) or autoimmune disease

(14%). 22% had liver carcinoma complicating the cirrhosis. Anti-HCV antibodies were found in 36%, among whom 72% had detectable viraemia.
On a declarative basis, vaccination rates for hepatitis A and B were 16% and 9%, respectively.
Prevalence of anti-HAV antibodies was 87.5%. Seromarkers of past and present infection with HBV was 32.4%: 12.5% had positive HBs antigen, 11.2% had HBs and HBc antibodies and 8.7% had isolated HBc antibodies. Only 19% had a vaccinated profile.
Thus, a total of 43 patients (55%) had an indication of vaccination against viral hepatitis: 4 (5%) patients presented an indication for HAV vaccination alone, 33 (42.5%) patients for HBV vaccination alone, and 6 (7.5%) patients for both vaccines. Vaccination proposal encountered no refusal.
Conclusion: In our cohort, we found high prevalence rates of HAV, HBV and HCV markers. However, vaccination against hepatitis A and B should be proposed to 12.5% and 50% of the patients respectively. These results support the recommendation that serologic testing for HAV and HBV should be done systematically in patients suffering from chronic liver diseases to propose a vaccination for hepatotropic viruses that could avoid an aggravation of their status.

P1594 Incidence of human papillomavirus genotypes and estimated repercussion of quadrivalent vaccine in Cadiz, Spain

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Objectives: Infection with human papillomavirus (HPV) as an essential cause of cervical cancer has now been established. The quadrivalent HPV vaccine protects against infections by HPV6, 11, 16 and 18.
HPV16 and HPV18 cause approximately 70% of cervical cancer worldwide so, the implementation of the HPV vaccine is a very important effort toward preventing high-grade cervical lesions.
The aim of the study was to know the incidence of different HPV genotypes and to estimate a possible repercussion of quadrivalent HPV vaccine in our sanitary area.
Methods: A total of 950 endocervical or vulvar swabs from woman suspicious of HPV infection (colposcopy and/or citology) were processed during 2006 to HPV detection. In 157 samples from 143 first visit patients (criteria for inclusion in the study) we detected any genotype of HPV. All samples were collected in duplicate and specimens were stored at $4^{\circ}C$ for a maximum of 6 days before detection. HPV extraction, amplification and detection were carried out according to the manufacturer (PVHfast, Genomica S.A., Madrid, Spain): a 450 bp fragment of L1 ORF region was amplified utilising the MY09/11 consensus primers in the thermocycler CeneAmp 2400. An internal control and endogenous human CFTR gene were co-amplified. Detection and typing of amplified products: RFLP in high resolution 2% agarose gel.
Results: There were 157 samples positives from 143 patients: 129 with one HPV genotype, 24 with two and four with more than two genotypes. The more frequents genotypes detected were HPV16 (25.2% of patients), HPV6 (22.4%), HPV61 (9.8%), HPV53 (9.1%) and HPV18 (7.7%). A total of 68 (47.5%) of the 143 patients had genotypes included in vaccine. Twenty-two patients (15.4%) had high grade cervical lesions, and 15 from these 22 patients (68.2%) associated with HPV16 and HPV18.
Conclusions:
1. The most frecuent HPV infection genotypes in our sanitary area were HPV16, HPV6 and HPV61.
2. The quadrivalent HPV vaccine will possibly prevent 47.5% of HPV infections by any genotype and 32.2% by high risk genotypes.
3. The reduction of HPV related CIN2, 3 or adenocarcinoma in situ is estimated at 68.2%.

P1595 Outbreak of measles in Catalonia (Spain): importance of laboratory confirmation

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Objectives: In 2001, Spain launched a measles elimination programme and in 2004 circulation of indigenous measles was interrupted. However, until world-wide eradication occurs, elimination maybe disrupted by epidemics in indigenous population as a result of an imported case. The objective of this study was to analyse microbiological and clinical characteristics of the measles outbreak that began in Catalonia at the end of 2006.
Methods: Suspected cases reported to the Department of Health, Generalitat of Catalonia, from 28 August 2006 to 18 June 2007 were studied. Laboratory confirmation was attained by determination of Measles-specific IgG and IgM antibodies by enzyme immunoassay and/or detection of the measles virus (MV) genome in urine with a multiple nested polymerase chain reaction (PCR) to detect the MV, rubella and parvovirus B19 viruses simultaneously. If PCR was positive for MV, confirmation was obtained by a measles-specific nested PCR.
Results: Of 534 cases involved, 372(69.7%) were confirmed. Laboratory confirmation was obtained in 327/372 cases (89.5%):137(137/327;42%) by MV IgM antibodies, 61 (61/327;19%) by MV genome PCR and 129 (129/327;39%)by both;38/372 (10%) were confirmed by epidemiological link and 7/372 (2%) were classified as clinically compatible due to lack of samples or evidence of direct contact with a confirmed case. Of 338 urine samples analysed (63.3% [338/534]), 190 (56.2%) were PCR positive and 114 genotyped to D4. 38/372 (10%) were confirmed by epidemiological link and 7/372 (2%) were classified as clinically compatible due to lack of samples or epidemiologic link to a confirmed case. Hospitalisation rate (HR) was 16.2% (60/372). Highest HR was observed in 26–35 years age group (28.8%). In 91 cases complications were present (24.5%), 35 of them were hospitalised. Most common complications were diarrhoea and/or vomiting (15.2%), otitis (5.4%) and pneumonia (2.1%). Complications were significantly greater in unvaccinated (85/151:56%) than in vaccinated cases (4/34:12%;$p < 0.001$. There were no deaths.
Conclusion: Laboratory confirmed cases accounted for 89.5%. Although laboratory confirmation is not essential during an outbreak, it reflects a sensitivity to disease involving not only laboratory and epidemiological surveillance units, but also attending physicians and should thus be encouraged. Thanks to the large number of clinical samples available for sequencing all cases could be set to belong to the outbreak.

Respiratory virology

P1596 Pilot evaluation of a commercial PCR-DNA microarray system (PneumoVir) for detection of respiratory viruses in clinical specimens

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Objectives: Diagnosis of respiratory virus infections is complicated by the large number of viruses implicated and the overlapping clinical presentations of these infections. Although nucleic acid amplification techniques offer increased sensitivity and improved standardisation relative to viral culture and antigen detection methods, their application to respiratory virus detection has been limited by the need to set up individual assays to detect each virus infection and the limited multiplexing capacity of real-time PCR. Application of DNA array technology provides an alternative solution to simultaneous detection of multiple viruses based on reverse hydridisation of targets amplified by multiplex PCR to an array of virus-specific probes. We therefore evaluated the PneumoVir Clinical Array (Genomica SA, Madrid) which has been designed to detect 17 respiratory virus types and subtypes in clinical specimens.

Methods: We performed beta testing retrospectively on 47 nasopharyngeal aspirate, sputum or bronchio-alveolar lavage samples which had been submitted for viral diagnosis during the 2006–7 winter season using the PneumoVir assay, and compared results with those of viral culture, direct immunofluorescence (DIF) for influenzavirus (IF) A and B, respiratory syncytial virus (RSV), parainfluenzavirus types 1–3 and adenovirus, and in-house real-time PCR for IF A and B, RSV and human metapneumovirus.
Results: One or more viruses were detected in 22, 23, 39 and 40 samples by viral culture, DIF, in-house PCR and the Pneumovir assay respectively. The PneumoVir assay detected dual infections in 13 samples and triple infections in 4 samples and gave concordant results for 19 of 21 viruses detected by viral culture, 22 of 23 by DIF, and 32 of 39 by in-house PCR. Discrepancies between PneumoVir and the other assays may reflect sample deterioration prior to the retrospective PneumoVir analysis, or differences in assay sensitivity and specificity.
Conclusions: The PneumoVir assay will be useful as a respiratory virus screen in view of its greater sensitivity than antigen detection and viral culture, and its greater multiplexing capacity than real-time PCR. The ability to detect multiple infections may enhance our understanding of the pathogenesis of respiratory tract infection.

P1597 Prospective evaluation of rapid antigen tests for diagnosis of respiratory syncytial virus and human metapneumovirus infections

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Objectives: Respiratory syncytial virus (RSV) and human metapneumovirus (hMPV) are two important viral pathogens causing respiratory tract infections in paediatric population. The use of rapid antigen detection tests allows for the prompt isolation and treatment of infected patients.
Methods: In a prospective study, using PCR as the "gold standard", we evaluated four rapid antigen tests including an EIA test for RSV (Directigen EZ RSV, Becton Dickinson, Sparks MD), a DFA test for RSV (Bartels, Trinity Biotech Carlsbad, CA) and two DFA tests for hMPV manufactured by DHI (Diagnostic Hybrids Inc. Athens OH) and Imagen (Oxoid, UK). We performed the 4 tests on 515 consecutive nasopharyngeal aspirates, which had adequate volumes and cells, submitted to the Clinical Microbiology Laboratory at Hartford Hospital from November 1, 2006 to April 21, 2007. Total nucleic acid was extracted by an easyMAG (bioMerieux) from each sample and was subjected to two user developed real-time TaqMan RT-PCR amplifications for RSV and hMPV.
Results: The TaqMan assays detected 238 (46%) RSV and 32 (6.2%) hMPV among all the submitted specimens. The rapid RSV EIA antigen assay was positive for 219 specimens, giving a sensitivity of 79.8% and a specificity of 89.5%. In contrast, RSV DFA was positive for 233 specimens with a sensitivity of 94.1% and a specificity of 96.8%. The DHI DFA detected hMPV in 21 specimens, giving a sensitivity of 62.5% and a specificity of 99.8%. All inconsistent results between PCR and antigen assays were confirmed with another RT-PCR directed to a second target in the RSV or hMPV gemone. The sensitivity, specificity, positive and negative predictive value for Imagen DFA for hMPV was 99%, 63.2%, 100% and 100% respectively.
Conclusion: We conclude while RSV EIA is user friendly it lacks sensitivity and specificity especially during off peak months. In contrast, RSV DFA is more sensitive and specific but is subjective and it demands technical time and expertise. Similarly, both hMPV DFA are highly specific in comparison to RT-PCR, but sensitivity awaits further improvement.

P1598 Use of a molecular panel in acute viral respiratory infections

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Objectives: The aetiology of acute influenza-like illness (ILI) and of other respiratory diseases generally is not established, as the diagnosis is mostly clinical, while laboratory tests for viruses are not diffused in routine laboratories. We used a molecular panel to detect common and emerging respiratory viruses in the upper respiratory tract from patients with ILI referred to our laboratory by general practitioners during the influenza season, or attending the respiratory disease clinic at the National Institute for Infectious Diseases in Rome. The predictive power and applicability of rapid influenza tests in the context of ILI was studied as well.
Methods: During February-April 2006 and 2007, 33 and 42 nasopharyngeal swabs were collected from patients with influenza-like illness. Moreover, 25 samples (4 nasopharyngeal swabs, 21 sputum samples) were obtained from individuals hospitalised for acute respiratory diseases during the period 30/11/06–11/4/07.
All the samples were analysed by PCR or RT-PCR for the presence of twelve different viruses (Influenza A and B, Metapneumovirus, Adenoviruses, Parainfluenza 1, 2 and 3, RSV, Coronaviruses OC43, 229E, NL63 and Rhinovirus). Moreover, nasopharyngeal swabs from ILI patients were analysed with rapid test for influenza A and B.
Results: The frequency of positive samples by PCR for at least one virus was 72.7% in 2006 and 59.5% in 2007; the rate of coinfections, generally including Rhinovirus, were 21.2% and 9.5%, respectively. By PCR, 11 influenza infections (33.3%, 9 A and 2 B) in 2006, and 13 in 2007 (30.9%, 9 A and 4 B) were identified. In 2007, 5 samples (11.9%) were positive to coronaviruses (3 OC43, 2 NL63), none in 2006. With rapid tests, only 6/24 (25%) influenza infections, all type A, were detected.
Concerning the hospitalised patients, all were positive for at least one virus, 4 (14.8%) presenting coinfection (3 with rhinovirus); 6 influenza infections were identified in 2007 (5 A and 1 B).
On the whole, the most commonly detected viruses were rhinovirus (44/100 samples, 44.0%), followed by influenza A (23.0%), generally H3N2.
Conclusions: Many viruses are frequently detected in respiratory samples from patients with acute respiratory illness. It is necessary to evaluate in a larger amount of samples and in distinct clinical contexts the clinical utility of the extended molecular panels for aetiologic diagnosis of acute respiratory illness, in order to confirm and/or exclude their viral aetiology.

P1599 Near-patient diagnosis of influenza virus infection in adult patients

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Objectives: Rapid and reliable diagnosis of influenza is essential for identification of contagious patients and effective patient management. Near-patient assays allow establishing the diagnosis within minutes, but were evaluated almost exclusively in young children. We evaluated near-patient assays in relation to the patient's age.
Methods: 194 patients with laboratory-confirmed influenza A virus infection diagnosed within a prospective cohort study were included. Cryopreserved nasopharyngeal swabs collected from these patients were tested by four near-patient assays (Binax Now Influenza A&B, Quick S-Influ A/B, Influ-A&B Respi-Strip, and Actim Influenza A&B). Main outcome measure was sensitivity of near-patient assays in relation to patient's age.
Results: The Binax Now, Quick S-Influ, Influ-A&B Respi-Strip, and Actim assays had an overall sensitivity of 19%, 18%, 26%, and 40%, respectively. The estimated sensitivity for influenza A virus detection in nasopharyngeal swabs was 17%-56% in children one year of age and

decreased to 8–22% in patients 80 years of age [logistic regression]. The sensitivity of Influ-A&B Respi-Strip and Actim decreased significantly with increasing age (P = 0.014 and P = 0.033, respectively [logistic regression]), a trend was observed for Binax Now (P = 0.074 [logistic regression]), and the low sensitivity of Quick S-Influ was similar in children and adults.

Conclusions: Less than one-fourth of influenza A virus infections may be identified in elderly patients using a near-patient assay. Consequently, near-patient assays are of limited value for confirming the clinically suspect diagnosis of influenza in adults. Antiviral therapy and additional diagnostic procedures cannot be withheld on basis of a negative near-patient test, particularly in adult patients.

P1600 Rapid detection and identification of 12 respiratory viruses by dual priming oligonucleotide system-based multiplex PCR assay

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Background: Acute respiratory virus infections are among the most common causes of human disease. Rapid and accurate diagnosis of a respiratory virus infection is important for providing focused and timely therapeutic intervention. Recently, a multiplex-PCR assay (Seeplex RV Detection, Seegene Inc., Seoul, Korea) was developed based on the dual priming oligonucleotide (DPO) system.

Methods: This study evaluated the Seeplex RV Detection assay for the simultaneous detection and identification of 12 respiratory viruses including parainfluenza viruses 1, 2, and 3, human metapneumovirus, human coronavirus 229E/NL6 and OC43, adenovirus, influenza viruses A and B, human respiratory syncytial viruses A and B, and human rhinovirus A using two primer mixes.

Results: The limit of detection (LOD) was estimated to be 10 copies/reaction per virus strain, and there was no cross-reactivity with common bacterial and viral pathogens. A comparison with conventional viral culture and immunofluorescence (VC/IF) method was carried out using 101 respiratory specimens from 92 patients. Using the VC/IF method, fifty-seven specimens (56.4%) showed positive results without any cases of co-infection. The Seeplex RV Detection assay identified the same viral strains in all 57 specimens. Seven out of the 57 specimens (12.3%) were found to be co-infected with other respiratory viruses, and 19 out of 44 (43.2%) specimens testing negative by VC/IF gave positive results by the Seeplex RV Detection assay.

Conclusion: Considering the high sensitivity and specificity, the Seeplex RV Detection assay might be a significant improvement over conventional methods for the detection of a broad spectrum of respiratory viruses.

P1601 Interest of a new technique of DNA chips for detection of respiratory viruses

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Objective: The aim of this study is to evaluate a multiplex RT-PCR combined with a new DNA-Chip hybrisation, comparing it to indirect immunofluorescence for detecting respiratory viruses.

Methods: We used 114 nasopharyngeal washing samples from our Hospital, each of them corresponding to different patients between November 2006 and January 2007. They were all between 2 months and 5 years old.

All the samples were performed: Indirect immunofluorescence (following protocol by Bartells©) and DNA Chips (Amersham Biosciences) for Respiratory Syncitial Virus, Adenovirus, Influenzavirus A and B and Parainfluenzavirus I, II and III, and Inmunochromatography for RSV (Binax©, Marnes la Coquette, France).

Those samples which resulted positive by, at least, two techniques, were considered true positives. The same was considered for true negatives.

Results: See table 1.

Conclusions: DNA chips avoid subjectivity in the interpretation of Immunofluorescence and are not affected by the tester's experience, as results reading is automated (BCS AiM Reader). However, it is a laborious technique with a higher duration than Immunofluorescence (about 6 hours vs the 1 and half which takes Immunofluorescence).

We obtained a very similar sensitivity and NPV values with both techniques close to 95%, but a better statistically significant ($p < 0.05$) specifity and PPV with DNA-chip.

Table 1.

	DNA chip				
IFI	RSV	Influenza A	Influenza B	Negative	Total
RSV	45	0	0	**6**	51
Inf A	0	2	0	0	2
Inf B	0	0	1	0	1
Negative	**4**	0	**1**	55	60
Total	49	2	2	61	114

P1602 Role of respiratory synctial virus compared to rhinovirus in acute respiratory illnesses in childhood

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Objectives: Acute respiratory illnesses (ARI) are a major cause of hospitalisation in childhood. Common techniques like immunofluoresence assay (IFA) and viral cultures do not detect all predominant pathogens. With a recently introduced sensitive technique, the real time polymerase chain reaction (RT-PCR), a specific diagnosis can be obtained within 24 hours. The objective of this study was to investigate the role of the most common respiratory viruses in ARI in childhood that lead to hospitalisation. Another objective was comparing the sensitivity of both RT-PCR an IFA in detecting viral pathogens of ARI in childhood.

Methods: Data were collected retrospectively from patients admitted between September 2006 and March 2007 who were hospitalised with acute respiratory symptoms at the Medisch Spectrum Twente in Enschede. Data collected included nasopharyngeal aspirate samples, clinical symptoms and for some patients biochemical parameters and X-rays. Virologic diagnosis was made by both RT-PCR and IFA.

Results: Of 149 patients included with ARI, viruses were detected in 80.5%. Most common were respiratory synctial virus (RSV) (42.3%) and rhinovirus (RV) (19.5%).

The only two significant clinical difference between these two major viral pathogens were a reduced food intake and fever in the RSV-positive patients. No significant differences were found between the RSV and RV groups regarding age, needed oxygen-therapy, length of hospital stay, the presence of infiltrates on chest X-rays, biochemical parameters and administration of antibiotics.

In 8 patients (14.8%) with a RSV, the IFA was false negative.

	Frequency	Percent
Rhinovirus	29	19.5
Human metapneumovirus	11	7.4
Respiratory syncytial virus	63	42.3
Adenovirus	2	1.3
Influenza virus A	6	4.0
Parainfluenzavirus 3	5	3.4
Parainfluenzavirus 2/4	2	1.3
Parainfluenzavirus 1	2	1.3
Negative	29	19.5
Total	149	100.0

Conclusion: Rhinovirus was the second most common virus after RSV in hospitalised infants. Except for food intake and fever, both viruses

showed the same clinical pattern. So although RSV is considered to be the most virulent viral pathogen in childhood, RV has the same virulence. The real time RT-PCR showed to be a more sensitive than the IFA technique to detect RSV infections.

P1603 Molecular epidemiology and disease severity of respiratory syncytial virus in relation to other potential pathogens in children hospitalised with acute respiratory infection in Jordan

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Objectives: To examine the molecular epidemiology of human respiratory syncytial virus (HRSV) in Jordan. To compare the disease severity of HRSV subgroups A and B and their associated genotypes in hospitalised Jordanian children with other potential respiratory pathogens.
Methods: Between December 2003 and May 2004, a total of 326 Jordanian children younger than 5 years of age and hospitalised with acute respiratory tract infections were recruited to the study. Total RNA and DNA were extracted from the collected nasopharyngeal aspirates. HRSV was detected by reverse transcription polymerase chain reaction (RT-PCR) using specific primers that target the nucleocapsid gene to produce a 278-bp amplicons. HRSV-positive strains were classified into subgroup A and B by restriction fragment length polymorphism analysis. Other respiratory pathogens were detected by RT-PCR and PCR according to previously published protocols.
Results: A total of 254 patients had at least one potential pathogen detected and 106 had mixed infections. 67 out of 140 HRSV-positive patients were co-infected. Significantly ($p < 0.0005$), more HRSV-infected children had severe disease compared to those uninfected with HRSV. Severe HRSV infections were significantly more likely to occur in those under 6 months of age ($p < 0.01$). There was no significant difference between the individual HRSV genotypes as potential causes of severe respiratory tract disease.
Conclusion: HRSV was the most frequent cause of severe acute respiratory infection in young children in Jordan. HRSV subgroup A was more predominant than subgroup B. Only four of the six previously described N genotypes were found among HRSV strains. Further studies over longer periods of time are warranted to better determine the epidemiology and disease severity of HRSV in Jordan.

P1604 Characterisation of viruses causing human respiratory infections via genomic identification for in vitro diagnosis. Clinical arrays/Clart PneumoVir

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Acute respiratory infections are among the most frequent diseases in our area, and they are one of the main reasons for consultation and hospitalisation. Due to a great variety of possible pathogenic agents and the high frequency of co-infections, it is necessary to use diagnostic methods that allow multiple, sensitive, and rapid identification of these viruses.
Objective: To evaluate the capacity of CLINICAL ARRAYS/ CLART®PneumoVir (CA-PV) for simultaneous detection of the 18 most frequent types of human viruses causing respiratory infections:influenza A H1-N1 virus (Flu-A, H1-N1), Flu A H3-N2, Flu-B, Flu-C, parainfluenza virus 1 (PIV-1), PIV-2, PIV-3, PIV-4A, PIV-4B, respiratory syncytial virus type A (RSV-A), RSV-B, human metapneumovirus A (hMPV-A), hMPV-B, rhinovirus, enterovirus, coronavirus, adenovirus, and bocavirus. Several Hospitals from Spain and UK have taken part in this multicentre study.
Method: Virus detection is performed via multiplex RT-PCR for amplification, and a new technology platform based on low-density micro-arrays (ArrayTube) for visualisation. This technology allows simultaneous detection of viruses and any necessary control in order to guarantee the reliability of the results obtained. In order to determine the diagnostic parameters of the kit, a comparative evaluation of CA-PV against clinical diagnostic's current methods (nested-multiplex PCR/agarose gel, culture) was made. A total of 374 clinical specimens were tested, being a true positive result judged according to the concordance between both methods. All the discrepancies were validated with sequencing, and homemade nested-PCR.
Results: 100% analytical sensitivity was obtained in the detection of 14 recombinant plasmids between 10 and 1000 copies. It was observed that there were no cases of unspecific detection of viruses obtaining 100% analytical specificity. About the diagnostic sensitivity and specificity, the behaviour of each virus after the validation of 374 clinical specimens showed that most of virus has sensitivity higher than 90%, and specificity higher than 98%.
Conclusion: CA-PV is useful in the clinical setting for rapid screening and detection of a panel of respiratory viral pathogens based on the following facts: (i) excellent specificities and sensitivities; (ii) rapid and automatic procedure; (iii) simultaneous detection allowing the recognition of co-infections, (iv) hMPV-A, hMPV-B, Flu-A H1N1, Flu-A H3N2, PIV-4A, and PIV-4B subtypes can be differentially detected.

P1605 Epidemiology of respiratory virus infections using xTAG RVP test

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Background: With the discovery of five new respiratory viruses since the year 2000 and advances in molecular technology allowing the detection of 20 respiratory viruses in a single test, we studied the epidemiology of respiratory virus infections.
Methods: A total of 1,060 nasopharyngeal specimens were collected from symptomatic patients and used in the study. Approximately 100 specimens per month, 50 paediatric (<20 yr) and 50 adult (>20 yr), were selected by randomised stratified sampling from specimens submitted to the Regional Virology Laboratory from November 2005 to October 2006. One half of the specimens were from paediatric patients (N=526) and one half were from adults (N=534). Total nucleic acid (DNA and RNA) was extracted using the MiniMag extractor (Biomerieux) and an aliquot was tested by the xTAG RVP Test from Luminex Molecular Diagnostics. The RVP assay is a multiplex PCR coupled to a fluid microarray that detects and identifies 20 respiratory virus types and subtypes representing 95% of all known respiratory viruses. A second aliquot was tested for Bocavirus using a separate PCR. Clinical information was collected by chart review. Results were analysed by month and by age group.
Results: Of the 1060 specimens tested, 424 (40%) were positive for one of 16 respiratory viruses including: 205 (19.3%) Rhino/Enterovirus, 58 (5.5%) Influenza type A or B, 45 (4.1%) Parainfluenza (types 1–4), 41 (3.9%) RSV (type A or B), 41 (3.9%) Metapneumovirus, 39 (39/947, 4.1%) Bocavirus, 20 Adenovirus, 14 Coronavirus (OC43, HKU1, NL63). Twenty five of 39 Bocavirus infections were dual infections. Only Rhino/Entero, RSV and Bocavirus were more prevalent in the <20 age group ($p < 0.05$). Influenza, Metapneumovirus and Coronavirus were only present in winter/spring months while Parainfluenza type 4 was only present in summer months; all others were distributed across most months. In January 2006, 10 viruses co-circulated in the community.
Conclusion: This study indicated the following: 1) Rhino/Enterovirus was the most prevalent virus infection of symptomatic children and adults, 2) only RSV, Rhino/Enteroviurs, and Bocavirus showed higher infection rates in children compared to adults, 3) 90% of dual infections (mostly Boca and Rhino/Enterovirus) occured in children summer, and 4) Influenza, Metapneumovirus and Coronavirus displayed a winter seasonality. The average rate of positives diagnosed per month for <20 yr group was 63% compared to 23% for adults.

P1606 **Bocavirus in hospitalised children in Italy**

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Objectives: Human bocavirus (HBoV), a novel respiratory agent identified in 2005, is now detected worldwide in paediatric as well as adult populations. However, in the absence of viral isolation and serological evidences and due to high percentages of co-infection with other respiratory viruses, its actual role in respiratory diseases is not yet clearly defined.
A molecular approach was undertaken to detect HBoV and nearly all known respiratory viruses, in archival and freshly collected respiratory samples from hospitalised children. In addition, HBoV was investigated in sera and convalescent samples from hospitalised children and in nasal washings from children affected by non-respiratory condition.
Methods: During November 2004 to May 2007, nasal washings from 415 children hospitalised with acute respiratory infection (ARI) and from 21 children hospitalised for non-respiratory illness were tested for the presence of an extensive range of respiratory viruses with molecular methods. Reverse transcription-PCR or PCR assays, followed by sequencing of the amplified fragments were undertaken to detect fourteen respiratory viruses: influenza A and B, respiratory syncytial virus (RSV), coronavirus OC43 and 229E, adenovirus, rhinovirus, parainfluenza viruses 1–3, human metapneumovirus, coronavirus NL63 and HKU1, HBoV. HBoV positive samples were subjected to Real Time PCR determination of HBoV specific viral load in nasal washings.
Results: Viral pathogens were detected in 214/415 cases (51.6%): RSV and rhinovirus positive samples were respectively 29.2% and 9.6% of cases. HBoV was the third most frequent agent (8.2%); 21/34 cases (61.8%) were in co-infection with another virus, mainly RSV. Children with HBoV as the sole pathogen presented pneumonia or bronchiolitis. Coinfection HBoV-RSV does not seem to worsen bronchiolitis (gravity score calculated as reported in Wainwright et al. N. Engl. J. Med. 2003). HBoV DNA copies in nasal washings were measured to correlate viral load with disease severity.
Interestingly, two children hospitalised for non-respiratory illness have been found positive to HBoV: one had gastro enteric symptoms and the other a clinical diagnosis of exanthema subitum.
Conclusion: This study is a confirming report of HBoV as a frequently detected respiratory agent and of its association with clinically important illnesses. HBoV high prevalence in young children does not seem to be due to persistence in individuals, but to its great circulation.

P1607 **Prevalence of respiratory pathogens in children hospitalised with lower respiratory tract infection in Greece**

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Objectives: Aim of the present study was to determine the prevalence of respiratory pathogens, including recently recognised viruses, in children hospitalised with lower respiratory tract infection (LRTI).
Methods: During September 2006 to August 2007, nasopharyngeal swab samples were collected from 375 previously healthy children, aged 14 days to 14 years old, admitted to paediatric wards of two hospitals in Greece, because of acute laryngotracheobronchitis, bronchiolitis, bronchitis or pneumonia. Genetic material was extracted and PCRs were performed for detection of *Mycoplasma pneumoniae*, respiratory syncytial virus (RSV), human metapneumovirus(hMPV), influenza viruses, adenoviruses, coronaviruses (HCoVs), human bocavirus (HBoV) and mimivirus.
Results: At least one aetiologic agent was detected in 37.06% of cases. Current infection due to *M. pneumoniae* was diagnosed in 11 (2.93%) children, aged 4 to13 years (with an exception of a 6 months old infant), presented mainly with bronchopneumonia or lobar pneumonia. RSV was detected in 61 (16.27%) children, aged 18 days to 3.5 years, while hMPV was detected in 4 (1.07%) children, aged 1.5 months to 1.5 years, most of them with acute bronchiolitis. Adenoviruses were detected in 26 (6.93%) children, aged 2 months to 7 years admitted for acute bronchiolitis or pneumonia. HBoV was detected in 13 (3.47%) children aged 2 months to 5 years with acute bronchiolitis, bronchopneumonia or laryngotracheobronchitis. Influenza viruses were detected in 10 (2.67%) children, aged 14 days to 6 years with bronchopneumonia and bronchiolitis. Coronaviruses were detected in 4 (1.07%) children, aged 2.5 months to 5 years. Mimivirus was not detected in any of the samples tested. Dual infections were identified in 10 (2.67%) children while one triple infection was also present.
Conclusions: Atypical pathogens and respiratory viruses are responsible for a large number of LRTI cases in children. RSV was the most common cause of viral lower respiratory tract infection while *M. pneumoniae* was a frequent cause of LRTI in children older than 5 years old, mainly presenting with pneumonia not associated with wheezing. Clinical characteristics of patients diagnosed with *M. pneumoniae*, RSV and adenovirus infection are relatively distinct. HBoV, a potential causative agent of LRTI, was frequently detected in preschool children with acute bronchiolitis or bronchopneumonia.

P1608 **Antiviral effect of a novel inhibitor of influenza virus haemagglutinin on influenza A (H5N1) virus**

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Background: The initial step of influenza virus infection is the attachment of the viral glycoprotein hemagglutinin to sialic acid-containing receptors of the host cells. Human and avian influenza A viruses differ in their recognition of host cell receptors: the former preferentially recognise receptors with saccharides terminating in Sia2–6Gal, whereas the latter prefer those ending in Sia2–3Gal. An attractive approach for the prevention of avian influenza infection involves inhibition of virus attachment to susceptible cells by synthetic analogs of cellular receptors on the basis of Sia2–3Gal. The goal of this investigation was to study the antiviral effect of a novel low-molecular polyvalent hemagglutinin inhibitor, containing Sia2–3Gal disaccharide motifs, on avian influenza A virus.
Methods: Low-molecular polyvalent sialoside was studied as viral hemagglutinin inhibitor of avian influenza A (H5N1, H5N2, H5N3) virus strains in the inhibition assays of virus binding with fetuin molecules (FBI) and infectious focus forming in MDCK cells. To investigate the protective effect of hemagglutinin inhibitor in an animal model, we have infected mice with highly pathogenic influenza virus strain A/Chicken/Suzdalka/2005 (H5N1), isolated from poultry and wild birds in Western Siberia. To study the development of viral resistance to novel inhibitor we have conducted serial influenza virus passages in MDCK cells in the presence of increasing concentrations of hemagglutinin inhibitor.
Results: The values of 50% inhibiting concentration (IC50) of hemagglutinin inhibitor obtained in MDCK cells and in FBI assay ranged from 1.5 to 10.0 microM for different influenza A virus strains. Intranasal administration of inhibitor (1.5 mg/kg) led to 90.5 ($\pm$5.2)% reduction of virus burden in the lungs of mice infected with highly pathogenic avian strain A/Chicken/Suzdalka/2005 (H5N1). The results of passaging experiments indicated that hemagglutinin inhibitor showed no tendency to induce viral resistance.
Conclusion: The data obtained in vitro and in vivo suggest that novel low-molecular polyvalent hemagglutinin inhibitor may be useful for the treatment of avian influenza virus infection.

Community-acquired infections – Adults

P1609 **A case of oto-mastoiditis caused by MRSA of porcine origin**

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Objectives: We report a case of a chronic oto-mastoiditis caused by a community-acquired meticillin resistant *S. aureus* (MRSA) in a Belgian woman living on a pig farm.

Methods: Samples from middle ear effusion and mastoid tissue were cultured following routine methods. Swabs taken from the throat, nose and perineum of the patient and from the nares of 13 pigs were cultured on a MRSA ID (Biomerieux) agar to detect MRSA. Four MRSA strains were send to the reference laboratory (Struelens, ULB, hospital Erasme) for detection of Panton Valentine leucocidin (PVL) and for characterisation by pulsed field gel electrophoresis (PFGE) and staphylococcal protein A (spa) typing.
Results: A 64-year old woman presented an oto-mastoiditis of the right ear with bone destruction at the roof of the middle ear cavity with concomitant cerebrospinal fluid leakage. Culture of middle ear effusion revealed MRSA. As the patient was living on a pig farm and several authors reported a link between the occurrence of MRSA in pigs and pathology in humans, samples of pigs were taken. Ten of 13 pig nares cultures and the patient's throat sample were MRSA positive. Four MRSA strains (3 pig origin, 1 patient origin) were send to the reference lab. They were PVL negative, non-typeable by PFGE and belonging to spa type t011, a type frequently isolated from pigs and pig farmers.
Conclusion: This case report describes a case of a severe complication of otitis media, caused by a community acquired, animal related MRSA strain.

P1610 Epidemiology of *Fusobacterium necrophorum* in university students

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Objectives: *Fusobacterium necrophorum* (FN) is the cause of Lemierre's disease, a life-threatening septicaemia preceded by an acute sore throat. The epidemiology of infection with this pathogen is unknown. We sought to clarify this by, examining throat swabs from, and questioning, 411 healthy university students; we also examined throat swabs from 103 patients who presented to local General Practitioners (GPs) with sore throat.
Methods: Throat swabs were collected between December 2005 and January 2006 and tested for FN and Epstein Barr Virus (EBV) DNA by Polymerase Chain Reaction, and cultured for beta-haemolytic streptococci (BHS).
Students were asked their age, gender, subject studied, occurrence of sore throat in the previous two weeks, episodes of sore throat over the previous six months, recent antibiotic treatment, smoking status, number of household members, number of people who shared their room at night, kissing (lip to lip) contacts in the past four weeks, frequency of contact with animals and animal type. Chi-squared and Fisher's exact tests using StatsDirect software were carried out to determine whether there were significant differences in frequencies between the *Fusobacterium*-positive and negative groups. Students who were positive for EBV or BHS were excluded from the epidemiological analysis of risk factors.
Results: Overall, 43/411 (10.5%) of the students were positive for FN, and these students were significantly more likely to have had 1 or more lip to lip kissing contacts in the previous 4 weeks than those who were negative (OR 3.22 [1.10–9.46]). None of the other variables were statistically significant in the univariate analysis. The number of FN-positive individuals was significantly higher in GP population who had a sore throat (20/103, 19.4%) than in the student population who had not had a sore throat in the preceding two weeks (29/304, 9.5%), OR 2.29 (1.23–4.25) $p=0.01$. There was an association with carriage of FN with EBV and BHS for both students (OR 2.99 [1.39–6.40] and GP patients (OR 3.15 [1.15–8.65])
Conclusion: Although we have previously failed to find the organism in older subjects, our findings indicate that throat carriage in healthy university students is common and related to lifestyle, being transmitted by intimate human physical contact. Our findings also support the hypothesis that *Fusobacterium necrophorum* is a more important cause of community-acquired sore throat than has hitherto been appreciated.

P1611 *Fusobacterium necrophorum* from sore throats and healthy controls

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Background and Objectives: Recently several publications have shown an association between clinical tonsillitis and *Fusobacterium necrophorum*. The objective of the study was to identify *F. necrophorum* by culture and Real-time PCR in throat swabs from patients with pharyngotonsillitis and healthy controls.
Methods: The isolation rate in 50 healthy military recruits aged 18 to 24 were compared with the rate in 70 randomly picked patients aged 16 to 30 years old from general practitioners with sore throats by using a selective anaerobic agar plate with, 5.0 mg/l nalidixic acid and 2.5 mg/l vancomycin and analysed by a species specific real-time PCR assay. All 1643 swabs sent to our laboratory in 2006 were examined for the presence of *F. necrophorum* using the same selective plate.
Results: *F. necrophorum* was found in 7 healthy controls and in 16 patients, $p > 0.05$, by culture and by PCR in 14 controls and 35, $p=0.03$. The mean Ct-value of PCR positive samples were significantly lower, $p=0.02$, in patients, 27 cycles compared to 31 cycles in controls.
During 12 month *F. necrophorum* was isolated from 146 throat swabs, giving an isolation rate of 9%. The highest isolation rate, 20%, was found in the patients aged 16 to 20 years, while only a few were found in children less than 10 years old and in adults older than 40 years old (<5%). A tendency towards a greater, but non-significant isolation rate in males were 10.4% compared to 7.9% for females, $p=0.08$). No variation in the isolation rate was observed through the year. There was a positive significant correlation between the isolation of *F. necrophorum* and the isolation of Group C streptococci ($p < 0.0001$). Contrary, the rate was significant lower when Group A streptococci was present ($p < 0.001$).
Conclusion: *F. necrophorum* is frequently a part of the normal throat flora. However this study also shows that larger quantities of *F. necrophorum* may cause tonsillitis, particularly in adolescents. However, as *F. necrophorum* can be present as normal throat flora the clinical symptoms have to be considered when considering *F. necrophorum* as the cause of tonsillitis. In addition, there seems to be an unexplained synergetic relationship between *F. necrophorum* and Group C streptococci.
We suggest that throat swabs from patients with a sore throat old negative in strep-A test should be examined anaerobically for the presence of *F. necrophorum*, particularly in patients 10 to 40 years old. Further studies are needed.

P1612 *Fusobacterium necrophorum* in acute severe ear, nose and throat infections

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Objectives: To determine the prevalence of *Fusobacterium necrophorum* in acute ear, nose, and throat (ENT) infections requiring hospitalisation, and to further characterise these infections in terms of age and gender distributions, associated inflammatory marker levels, and the effect of pre-admission antibiotics on culture results.
Methods: A review was performed of all acute infectious cases admitted to the ENT department at Aarhus University Hospital, Denmark, between January 1st, 2001 and December 31st, 2006. Patient age and gender, use of pre-admission antibiotics and microbiological findings were recorded. C-reactive protein levels (CRP), leukocyte counts, and erythrocyte sedimentation rate (ESR) at admission were also noted.
Results: *F. necrophorum* (13.6%, 195/1430) and Group A *Streptococcus* (13.7%, 196/1430) were the most frequent isolates. *F. necrophorum* was primarily isolated from cases of peritonsillar abscess (90.8%, 177/195). It was also found in several cases of tonsillitis, cutaneous neck abscess, retro- and parapharyngeal abscess. *F. necrophorum* was less frequently isolated from patients who received pre-admission antibiotics compared to those who did not (13.7%, 53/387 versus 17.6%, 129/732). Of patients that received pre-admission antibiotics, 85.2% (46/54) received penicillin. All *F. necrophorum* isolates were susceptible to

penicillin and metronidazole. The majority of *F. necrophorum* infections occurred in patients between the ages of 10 to 29 (82.5%, 160/194). The ratio of males to females was 1.3:1. Females tended to have *F. necrophorum* infections at a younger age than males (female median age = 18, male median age = 21, p = 0.007). *F. necrophorum* infections were associated with a significantly higher CRP value than infections with *Staphylococcus aureus* (p < 0.001), non-hemolytic streptococcus (p = 0.037), and *Streptococcus pneumoniae* (p = 0.006), but not Group A streptococcus (p= 0.116). This pattern was not noted for leukocyte counts or ESR, although values were in the upper range.
Conclusions: This study suggests that *F. necrophorum* is a far more widespread pathogen in severe acute ENT infections than previously reported. The age and gender distributions suggest increased susceptibility to the bacterium in puberty and early adulthood. We report an elevated CRP value for *F. necrophorum* infections compared to infections with other common ENT pathogens, however, the clinical significance of this remains to be elucidated.

P1613 Trends in listeriosis in a university hospital (1983–2007)

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Infection caused by *Listeria* is acquired by the ingestion of contaminated food which has increased in recent years due to the frequent use of ready-made meals. This, together with a longer life expectancy of patients with chronic diseases and a large number of immunosuppressed patients, are factors which may explain the apparent increase in the incidence of listeriosis.
Objectives: To know the risk factors and clinical aspects of infection by *Listeria* and evaluate epidemiological changes over time.
Methods: We retrospectively reviewed the cases of listeriosis reported in the Department of Microbiology from 1983 to 2007. Patients were arbitrarily classified into 2 groups: group 1 from 1983 to 1995 and group 2: from 1996 to 2007.
Results: 72 patients (21 group1; 49 group 2) with listeriosis were included. 56.9% (41/72) were males with a mean age of 53.8 years (0–87). The mean age rose significantly in group 2. 10% (7/70) were hospital-acquired, being significantly greater in group 1.Underlying diseases were present in 81.9% (59/72), including chronic liver disease (34.3%), alcoholism (28.6%) and cancer (28.6%) and with a significant increase in heart diseases and cancer and a decrease in HIV patients in group 2. 30% (21/70) and 28.5% (20/70) were receiving corticoids and immunosuppressive therapy respectively, with an increase, albeit not significant, in group 2. Previous diarrhoea was reported in 7 cases (10%). 7.4% (5/68) were pregnant women, 83.6% (56/68) presented with sepsis, 44.8% (30/67) had meningoencephalitis and 7.3% (5/) focal infections. Blood culture was positive in 79.1% (57/72) as was CSF culture in 40.2% (29/72). Serotype 4 was the most prevalent but a significant increase in serotype 1 was observed in group 2. 20.6% (14/68) required ICU admission and mortality was 20.9%, with a non significant increase in group 2.
Conclusions: Cases of listeriosis have increased in the last decade due to a rise in sepsis. At present, chronic liver disease continues to be the most important risk factor, although an increase in the number of cases with heart disease and cancer receiving immunosuppressive therapy is of note. In the last decade, mortality has shown a trend to an increase and has reached a value of 24%.

P1614 Clinical characteristics of *Eikenella corrodens* infections: a 10-year experience in a tertiary hospital

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Objectives: *Eikenella corrodens* is a show-growing, gram-negative bacillus, difficult to isolate. It is commonly found in oral, gastrointestinal, respiratory and, more rarely, in genitourinary flora. Head and neck infections and those related with human bite wounds are the most frequent sites of *Eikenella* infections reported in the literature.
Methods: Descriptive retrospective study from January 1996 to February 2006 in a 700 bed tertiary hospital. All in-patients with infections where *Eikenella* was isolated from a clinical specimen were analysed.
Age, sex, predisposing factors, main diagnosis, infection site, others isolated organisms, antibiotic sensitivity, antibiotic therapy and outcome were recorded.
Results: Thirteen patients were identified during the study period. Nine were male (69%), the mean age was 61 years (range: 3 months to 75 years). Predisposing factors were: 3 patients had synthetic materials (1 abdominal-aortic bypass, 1 dura mater implant, 1 intrauterine device). 3 had undergone surgical procedures, 1 patient was an intravenous drug user and another one was an oncology patient.
Eleven patients (85%) had extra oral infections: 4 surgical site infections, 2 cardiovascular infections, 1 gastrointestinal tract, 2 pneumonia, 1 sepsis and 1 skin infections.
Four patients had monomicrobial isolates and 9 polymicrobial. In polymicrobial cases the most frequent isolated pathogens were: 67% *Streptococcus viridans*, 11% *Pseudomonas*, 11% *E. coli* and others in 33%.
All the *Eikenella* strains were sensitive to penicillin G, amoxicillin-clavulanic, cefotaxime and fluoroquinolones. All patients required a surgical procedure in addition to antibiotic therapy, but in two cases only drainage was made (canaliculitis and peritonsillar abscess). One patient received only one antibiotic and twelve 2 or more drugs (6 betalactams, 5 amino glycosides, 3 lyncosamines, 3 nitroimidazoles, 2 glycopeptides, 2 quinolones, 1 tetracyclines). All the patients had a favourable clinical outcome of the infection. One patient died of an unrelated cause.
Conclusions: In this study extra oral locations were more frequent than previously reported. In 61% of patients one or more predisposing factors for this infection were identified. Most of patients had mixed infections. *Eikenella* antibiotic sensitivity was similar to that reported in literature. 69% of cases required surgical procedures in addition to the antibiotic therapy. Outcomes were favourable in all patients.

P1615 Time changes in microbiology of diabetic foot ulcers in a diabetes clinic, Athens, Greece

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Objectives: Infection of the diabetic foot consists a major cause of morbidity and amputation in diabetic persons. Aim of this study was to record the microbiology of diabetic foot ulcers and the susceptibility of isolates to commonly used antibiotics.
Methods: 4-year (2003–2006) retrospective study of 125 consequent cultures (75 diabetic persons with foot ulcers, Diabetes Outpatient Clinic and/or Internal Medicine wards).
Results: 88/125 cultures (70.4%) were polymicrobial (2–6 isolates/culture), 36 monomicrobial and 1 sterile (median: 2 isolates/culture). Most common microorganisms isolated were coagulase (–) staphylococcus (CNS, 42.4% of cultures), *Enterococcus* spp. (34.8%), *Pseudomonas* spp. (33.6%), *S. aureus* (32.8%), *Proteus* spp. (12.8%). Aerobic Gram(+) bacteria were isolated in 84.8% of cultures, while aerobic Gram(–) in 63.2% and anaerobes in 8.8%. In an older study of ours (1992–1996), Gram(+) and Gram(–) aerobes were isolated in 65% and 80%, respectively. Meticillin-resistant were 31.7% of *S. aureus* and 75.2% of CNS isolates, while 1/43 *Enterococcus* isolate was VRE. 16.8% of *Pseudomonas* isolates were imipenem resistant. In our older study, 50% of *Staphylococcus* isolates were meticillin-resistant and 23% of *Pseudomonas* isolates were imipenem-resistant.
Conclusions: (1) Cultures were mostly polymicrobial. (2) Gram(+) cocci were more common than Gram(–) rods, contrary to our previous findings 10 years ago. (3) Staphylococci and enterococci were the most common isolates among Gram(+) and *Pseudomonas* spp. among Gram(–) bacteria.

P1616 Biodiversity of UTI pathogens in diabetics

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Introduction: Urinary tract infection (UTI) is a significant health problem affecting people worldwide. Women are especially prone to UTI for reasons which are poorly understood, common factor known is the shortness of urethra which contributes to the easy passage of uropathogens into the bladder. Any abnormality in the urinary tract that obstructs the flow of the urine sets a stage for infection.

Diabetic patients have a higher risk of acquiring UTI because of the changes in the immune system. Any disorder that suppresses the immune system like diabetes raises a risk of UTI.Some of them are potentially lethal like mucormycosis which never affects people without diabetes. Urinary tract is also a cause of bactraemia among patients with diabetes, it has been established that uropathogens like *E. coli* grow better in urine after the addition of glucose in various levels. Many people with diabetes have dysfunctional bladders which contract poorly & hence allows the urine to remain stagnant for longer periods thus providing luxorious ponds for bacteria to grow in.

The current study is based on:

- The incidence of UTI in diabetic patients.
- To detect most prevalent strains of uropathogens.
- To develop link between diabetes and lower socio economic group.
- Risk factors associated with UTI in diabetic patients.
- Screening of MDR strains.

Methods: The prospective study involved collection of urine samples from diabetic patients belonging to higher & lower socioeconomic group. Urinalysis and culturing methods were followed to isolate uropathogens from the samples collected. Confirmation of possible UTI was achieved through viable colony counts in CFU/ml of the specimen.

Analysis of C-Reactive protein was performed in patients with diabetes, without diabetes, and in patients with diabetes but no UTI.

Clinically significant uropathogens were checked for resistance hence isolation of some MDR strains was established.

Isolation of plasmids was performed in these strains to detect specific mutations associated with diabetes induced UTI.

Experiments were also carried out for the estimation of iron in diabetic blood samples so as to corelate the association of these factors with UTI.

Results: 500 patients were evaluated for the study, and the results indicated 46.4% people had diabetes. *Enterococcus faecalis* 25%, *Pseudomonas aeruginosa* 15.94%, E. coli 7.4%, *Klebsiella* 3% & *Staphylococcus* 10.34% were found to be frequently encountered UTI pathogens.

Conclusion: Prevalence of UTI is higher in diabetic patients.

P1617 *Shigella*/enteroinvasive *E. coli* infections in the county of Frederiksborg, Denmark

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Objectives: To describe epidemiological and microbiological characteristics of *Shigella*/Enteroinvasive *E. coli* (EIEC) infections over a five-year period in a Danish county from 2003 to 2007.

Methods: Stools from patients with gastroenteritis in Frederiksborg county with 370,000 inhabitants comprising 7% of the Danish population were examined for enteropathogenic bacteria, including *Shigella* spp. and EIEC. Examinations were done at the National Reference Center at Statens Serum Institut, Copenhagen, in 2003 and until April 1, 2004. From that date until the present the cultures were examined by the local microbiology laboratory at Hillerød Hospital. From September 1, 2006, culturing was supplemented by a LightCycler real time PCR of virulence genes of diarrhoeagenic *E. coli* including ipah, characteristic for both *Shigella* spp. and EIEC. Detection of production of extended-spectrum β-lactamases (ESBL) and production of AmpC β-lactamase was done by a combined disk method.

Results: The number of EIEC infections varied between 0 and 10 annually, while the number of *Shigella*/EIEC infections fluctuated between 15 and >60. The percentage of *Shigella*/EIEC infections diagnosed in our county compared to the whole country increased gradually from 8% in 2003 to approximately 16% in 2007. *Shigella* spp. comprised mainly *Shigella sonnei*. The high number of infections in 2007 was mainly due to a national outbreak with *S. sonnei* in August, 2007, where the source was determined by Statens Serum Institute's national surveillance team to be imported baby corn from Thailand. The outbreak included one case of bacteraemia. The epidemic strain was sensitive to ciprofloxacin and mecillinam, but resistant to ampicillin, trimetoprim, sulphonamides and cefuroxime. The strain produced AmpC, but not ESBLs.

Conclusions: Gastroenteritis with *Shigella* spp. usually occurs sporadically in our part of Denmark. An exception was an outbreak with *S. sonnei* in 2007 which was unusual because the epidemic strain produced AmpC and because one patient was found with bacteraemia. The increasing number of *Shigella*/EIEC infections compared to the rest of Denmark could be due to the extensive traveling done by the inhabitants of our county and/or the use of a sensitive diagnostic method.

P1618 *Aeromonas* infection: clinical observations and laboratory characteristics of an 82-case French study

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Objectives: to describe characteristics of current human Aeromonosis in a European country (France).

Methods: A 6-month period prospective study was conducted among a 70-French nonteaching hospital network (may-october 2006). In case of *Aeromonas* isolation from biological samples, clinical data and laboratory findings were recorded and strain(s) collected for partial rpoB gene sequence based identification and antimicrobial susceptibility testing (disk diffusion testing).

Results: 99 patients were included, of whom 82 were infected (median age: 56 years, sex ratio: 1.6) and diseases were distributed as follows: wound infections (43%), bacteraemia (24%), gastroenteritis (19%), respiratory disease (7%), peritonitis (4%), ocular infection (1%) and urinary tract infection (1%). Underlying conditions were found in 35% (malignancy: 55%, diabete mellitus: 20%, hepatobiliary diseases: 14%), wounds in 36% and an environmental exposition in 44%, mainly clustered in the wound infection group, with telluric-related illnesses being as prevalent as water-related illnesses. Variations in data according to the type of infection were noted.

Among 76 strains analysed, species were distributed as follows: *Aeromonas veronii* (29), *A. hydrophila* (26), *A. caviae* (17), *A jandei* (2), *A. allosaccharophila* (1), *A. bivalvium* (1). Distribution varied greatly with the type of infection.

Proportion of susceptible strains were: amoxicillin 3%, co-amoxiclav 18%, cefalotin 47%, imipenem 67%, cefotaxim 96%, nalidixic acid 86%, ofloxacin 91%, tobramycin 80%, gentamicin 100% and tetracyclin 89%. Rates differed markedly between species. Two strains were found to bear extended spectrum betalactamase (ESBL) resistance.

Conclusion: this prospective study gives detailed and updated information on *Aeromonas* infection diseases and their management. As a matter of fact, the most prevalent infection is currently wound infections, followed by bacteraemia, enteritis and emerging respiratory tract infections.

P1619 The changes of neutrophil activation markers (CD11A, CD11B, CD62L, CD66B), serum neutrophil elastase levels after the operation period, and its relationship with pre-operative metabolic control parameters in type 2 diabetic patients

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Aims and Background: The aim of this study was to evaluate the changes of neutrophil activation markers (CD11A, CD11B, CD62L, CD66B), serum neutrophil elastase levels after the operation period, and its relationship with pre-operative metabolic control parameters in type 2 diabetic patients.

Materials and Methods: Twenty-five type 2 diabetic patients (aged 60.2 ±12.4 years, BMI: 29.5±5.1 kg/m^2) and 14 non-diabetic healthy controls (aged 51.4 ±16.8 years, BMI: 25.7±5.4 kg/m^2) were enrolled in the study. Serum neutrophil elastase levels was determined by ELISA method. The neutrophil integrin expression assay were performed by flow-cytometry as a unit of relative fluorescence intensity (RFI). Samples were analysed on a FACSCalibur flow cytometer (Becton Dickinson). The data was analysed using CellQuest software (BD Biosciences).
Results: The study results showed that there were statistically significant difference between study patients (type 2 diabetic patients) and control subjects in accordance to preoperative CD11a expression (RFI) (17.3±2.9l vs 19.8 ± 3.6, $p<0.05$), preoperative CD11b expression (RFI) (24.9±7.9 vs 34.2 ± 5.9, $p<0.01$). There were no statistically significant difference between study patients (type 2 diabetic patients) and control subjects in accordance to preoperative CD62L expression (RFI) (27.8±5.8 vs 31.3±9.5, $p>0.05$), preoperative CD66b expression (RFI) (4.5±1.5 vs 4.4 ± 1.1, $p>0.05$), preoperative serum neutrophil elastase levels (ng/ml) (0.4±0.3 vs 0.4±0.2, $p>0.05$). There were statistically significant difference between pre and post operative period CD11a, CD11b, CD66b expression, serum neutrophil elastase levels in type 2 diabetic patients. The correlation analyses (Pearson) have shown that in diabetic subjects, there was a statistically significant correlation between preopCD11a expression and preopCD11b expression ($r=0.55$, $p<0.01$), preopCD11a expression and preopCD62L expression ($r=0.51$, $p<0.01$), preCD11b expression and preNE level ($r=-0.41$, $p<0.05$), postopCD11a expression and postopCD11b expression ($r=0.42$, $p<0.05$), postCD11b expression and postCD66b expression ($r=0.55$, $p<0.01$), postCD62L expression and postNE level ($r=0.43$, $p<0.05$).
Conclusion: These results have shown that there may be a relationship between neutrophil activation and postoperative diabetic complications. This relationship may be important for developing infectious and other related complications at postoperative period in type 2 diabetic patients

P1620 Demographic, clinical and laboratory data in 232 typhoid fever cases

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Objective: It is estimated that ~21,650,000 episodes of typhoid fever and 216,500 deaths due to the disease occurred in areas of endemicity in 2000. The risk of acquiring typhoid fever is increased among travelers to regions where the disease is endemic. 90% of cases occur each year in Asia. In this study we review clinical and laboratory aspects of typhoid fever admitting in a teaching hospital in a nine years period.
Methods: This is a retrospective descriptive study performed upon patients with definite diagnosis of typhoid fever (positive blood or bone marrow culture for *S. typhi*) in St. Zahra Hospital, Isfahan (centre of Iran) during 1997 till 2006. The subjects were comprised 232 patients [170 (73.3%) males and 62(26.7%) females aged 1–40 years. The data were achieved from medical records and analysed by using SSPS software.
Results: Upon this study, 170 (73.3%) were males and 62 (26.7%) females and among them 141 (60.75%) were aged between 10–30 years. Clinical signs and symptoms: fever 228 (98.2%), headaches 170 (73.2%), nausea and vomiting 117 (33.6%), abdominal pain 101 (43.5%), diarrhoea 101 (43.5%), constipation 97 (41.8%), myalgia 64 (27.5%) sore throat 48 (20.6%), dizziness 36 (15.5%), epistaxis 17 (7.3%), psychic symptoms 11 (4.7%), hematochezia 7 (3.01%), convulsion 2 (0.8%), splenomegaly 103 (44.3%), rose spots 52 (22.4%), abdominal tenderness and crepitation 4 (1.8%), hepatomegaly 32 (13.7%), cervical lymphadenopathy 25 (10.7%) pulmonary rales 19 (8.1%) relative bradycardia 12 (5.1%), meningeal irritation 3 (1.2%). Para clinical data: positive widal test 158 (68.1%), anaemia 156 (67.2%), leucopenia 111 (47.8%), Transaminase elevation 76 (32.7%), thrombocytopenia 45 (19.3%), leukocytosis 44 (18.2%). Mortality rate 1 (0.4%) an Afghan refuges due to septic shock.
Conclusion: The clinical and laboratory data's in typhoid fever are nonspecific, it occurs mainly in young males, serology is helpful but culture is the definite method for diagnosis. It needs a high index of suspicion in a patient with prolonged fever who had travel to endemic areas.

P1621 Risk factors for acute erysipelas: a case-control study

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Objectives: To analyse clinical risk factors for acute erysipelas (non-necrotising bacterial cellulitis).
Methods: From March 2004 to March 2005, 90 patients with 98 disease episodes presenting with acute erysipelas at two hospitals in Tampere, Finland were recruited in the study. For each patient, one age and sex matched control subject was recruited from the population of Tampere. The patients and the controls were interviewed and clinically examined. Conditional logistic regression analysis was used to identify risk factors.
Results: Fifty-eight of the 90 patients (64%) were men. The median age was 58 years (range 21–90). Six patients were recruited 2 or 3 times because of recurrence during the study period. Erysipelas was localised in the lower extremity in 84, in the upper extremity in 7 and in the face in 7 episodes. In a multivariate analysis, the body mass index of ⩽30 [OR 5.4 (95% confidence interval 1.5–20.4)], disruption of the cutaneous barrier [8.2 (2.5–26.2)] and chronic oedema of the extremity [12.0 (1.1–125.8)] were found as independent risk factors for acute erysipelas whereas diabetes, malignant or cardiovascular diseases, smoking, alcoholism or a previous surgical procedure of the ipsilateral extremity were not. Forty-four (49%) patients had a positive history (PH) of at least one erysipelas episode before entering the study. The median age of these patients did not differ from patients with a negative history (NH) of a previous erysipelas episode. PH patients had been significantly younger at the time of their first erysipelas episode (49 years, range 12–78) than NH patients (58 years, range 28–84, $p=0.004$). We also compared the general and local risk factors of PH and NH patients. Obesity, toe-web intertrigo and previous surgical procedures of the ipsilateral extremity were more common in PH patients and a recent (<1 month) traumatic wound in NH patients, respectively.
Conclusion: Previous studies using hospitalised control patients have found obesity, chronic oedema and disruption of the cutaneous barrier, especially toe-web intertrigo as independent risk factors for acute erysipelas. Our study confirms these findings by using population controls. Obesity and toe-web intertrigo seem to predispose also for recurrent erysipelas but other, possibly inherited, factors may also play a role.

P1622 Spontaneous vertebral osteomyelitis: comparison of cases without a microbiological diagnosis to definite pyogenic and tuberculous cases

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Objectives: Vertebral osteomyelitis is an uncommon but serious entity, whose aetiologic diagnosis is not always possible. Few data are available on outcome of cases without a microbiological diagnosis (possible vertebral osteomyelitis). The aim of this study was to analyse features, diagnostic approach, management, and outcome of spontaneous vertebral osteomyelitis cases in adults.
Methods: We performed a retrospective review of 82 cases of vertebral osteomyelitis presenting over a 11-year period (from 1995 to 2005) to 2 Italian tertiary hospitals. All patients had a strict case definition. We compared data in patients with vertebral osteomyelitis due to various aetiologies and possible vertebral osteomyelitis.
Results: A total of 82 patients met diagnostic criteria for vertebral osteomyelitis, giving an incidence of 0.83/100,000 inhabitants/year. The diagnostic yields of blood culture, CT-guided percutaneous needle biopsy of spine, and surgical culture were 43.6%, 72.7%, and 91.6%, respectively. Finally, a causative organism was identified in 60 (73.2%) of 82 cases. *Mycobacterium tuberculosis* was the organism most frequently responsible for the illness, followed by *Staphylococcus aureus*, streptococci, and Gram-negative bacilli. The median diagnostic delay

was longer in tuberculous cases (4 months) than in pyogenic (1 month) and possible vertebral osteomyelitis cases (0.5 month) ($P < 0.017$). All patients received antibiotic treatment, and 21 (25.5%) of them underwent additional surgical treatment. This was required more frequently in tuberculous cases (40.7%) than in pyogenic (25.0%) and possible cases (9.1%) ($P < 0.028$). Recovery was more frequent in pyogenic (78.1%) and possible cases (81.8%) than in tuberculous cases (30.8%) ($P = 0.26$). Of 67 patients who completed the 1-year follow-up period, 2 relapsed, 3 died and 24 had persisting painful disability. The latter finding was more frequent in tuberculous cases (66.7%) than in pyogenic (21.7%) and possible cases (15.0%) ($P < 0.03$).
Conclusion: Nearly a fourth of spontaneous vertebral osteomyelitis cases does not achieve a microbiological diagnosis even after undergoing invasive diagnostic methods. However, possible vertebral osteomyelitis cases treated at our institutions have both diagnostic delay and outcome similar to that shown by pyogenic cases. The challenge continues to be the earlier recognition of tuberculous vertebral osteomyelitis that is associated to the highest risk of long-term morbidity.

P1623 Change from oral antidiabetic therapy to insulin and risk of urinary tract infections in type 2 diabetic patients: a population-based prescription study

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Objective: Diabetes is a risk factor for urinary tract infections (UTI). This study assessed whether a change from oral antidiabetic drug (OAD) to insulin therapy with improved glycemic control decreased the rate of antibiotic prescriptions for UTI among Type 2 diabetics.
Method: We used population-based healthcare databases to identify 2,843 Type 2 diabetic patients who switched from OAD to insulin therapy. Each patient was observed for 365 days before and after the change. Episodes of UTI were defined as filled prescriptions for a UTI-specific antibiotic. We used conditional logistic regression to estimate the relative risk (odds ratio (OR)) of having at least one UTI in the insulin vs. OAD period, both overall and stratified by improvements in glycemic control.
Results: The overall period prevalence of UTI was 15.6% in the OAD period and 15.9% in the insulin period. The OR for a UTI episode in the insulin period compared to the OAD period was 1.05 (95% CI 0.87–1.26). After the change to insulin therapy, 53% of all patients experienced a decrease in mean HbA1c level (median decrease=1.5%, interquartile range 0.93%-2.30%). Stratifying by gender, we found a period prevalence of 23.3% among women and 7.3% among men in the OAD period, compared to 24.0% among women and 8.2% among men in the insulin period. The corresponding ORs were 0.98 (95% CI: 0.78–1.22) for women and 1.25 (95% CI: 0.87–1.79) for men. The most pronounced effect of insulin therapy initiation on UTI risk was observed for patients of both genders with a high baseline comorbidity level, a history of UTI treatment, and age less than 65 years at study entry (ORs between 0.67 and 0.85). However, statistical precision was limited
We did not find a consistent effect of level of glycemic improvement on UTI risk.
Conclusion: Initiation of insulin treatment with or without tightened glycemic control had no impact on risk of treated UTI in Type 2 diabetic patients.

P1624 Spatial correlation among antimicrobial usage density and resistance in *Escherichia coli* community urinary tract infections: EUREQA Project report

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Objective: Explore space-time correlations among antimicrobial usage density and resistance in community urinary tract infections (cUTIs) caused by *E. coli* within the city of São Paulo, Brazil, as part of the EUREQA Project.
Methods: EUREQA is a multi-phase spatial and temporal project aimed at interpreting bacterial infections and resistance dynamics and their correlations with risk factors, mainly antimicrobial usage density. Routine outpatient urine culture results for *E. coli* (single isolate per culture, with $\geqslant$100,000 CFU/ml) between 2002 and 2006 were considered. In order to detect resistance clusters, cases were categorised in susceptible (S) or resistant/intermediate (R) to Ciprofloxacin (CIP), Nitrofurantoin (NIT) and Trimethoprim–sulfamethoxazole (STX). Cases were then geocoded in a digital map and Kernel function method was used to detect clusters (TerraView 3.2, INPE). Additionally, antimicrobial usage density (IMS Health, Brazil) was geocoded according to consumption location origin. Comparison unit was defined as DDD/1000 inhabitants-day. Exploratory spatial correlation was based on Kernel patterns of resistance and antimicrobial DDDs, to explore possible concomitant hot spots formations.
Results: 29,199 urine cultures were geocoded during the period, with R to CIP 15%, NIT 4.6%, and STX 32.5% in the 5 years altogether. A non-random pattern (hot-spot) of total sample *E. coli* was observed in one south-central district repeatedly every year. Although the same district also presented a hot-spot for CIP, NIT, and STX R *E. coli*, other hot-spots of R *E. coli* were observed in adjacent districts, probably meaning different risk factors for resistance acquisition and/or spread. Additionally, hot-spots for CIP, NIT and STX consumption were located in a larger district influence area, including the ones where resistance occurred.
Conclusions: Kernel smoothing function was the initial spatial data exploratory technique applied for identifying evidences for spatial clusters. Evidence for independent resistance spatially aggregated was detected. Antimicrobial consumption presented a similar geographical pattern and could not be ruled out as a risk factor for resistance development. However, other methods must be applied to further quantify and confirm this hypothesis. Geographical information systems, with spatial and temporal methods, are useful tools for better understanding and curbing resistance by detecting target areas for public health interventions.

P1625 Comparison of phylogroups and virulence genes of *Escherichia coli* obtained from healthy humans and patients with urinary tract infection in Denmark

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Objectives: *Escherichia coli* (Ec) causing urinary tract infections (UTI) in women is believed to stem from the patients own faecal flora. Ec causing UTI often belong to phylogroup B2, which is more virulent than the other common phylogroups. The objective was to investigate the phylogroup distribution and the presence of selected virulence genes (VGs) often detected in extra-intestinal pathogenic Ec of Ec isolates from healthy humans and patients with UTI in Denmark.
Methods: In 2004, 109 Ec faecal isolates were obtained from healthy humans as part of the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP). Furthermore, 131 Ec urine isolates were collected from Danish patients with UTI in 2005. All isolates were investigated by three multiplex PCR reactions for their phylogenetic background (A, B1, B2, D) and presence of eight VGs (kpsM II, iutA, papA, papC, hlyD, sfaS, focG, afa). Results were analysed using Chi-square, Fisher's exact and Mann-Whitney test.
Results: Healthy human isolates grouped into phylogroups A (24%), B1 (16%), B2 (36%) and D (25%) and patient isolates grouped into phylogroups A (22%), B1 (7%), B2 (47%) and D (24%). B1 isolates were more prevalent in healthy humans than patients ($p = 0.0371$). Similar prevalences of group A, B2 and D isolates from healthy humans and patients were observed.
The 109 healthy human and the 131 patient isolates had similar distributions of the VGs (Table 1).
B2 isolates from both groups carried from 0–6 VGs. The median no. of VGs carried by B2 isolates were highest in isolates from patients

(3.56 VGs/isolate) compared to isolates from healthy humans (2.63 VGs/isolate) (p = 0.0014). B2 isolates with iutA, papA, papC and hlyD were significantly more prevalent among patient isolates (Table 1). B2 isolates from both healthy humans and patients carried significantly more VGs compared to phylogroups A, B1 and D.
Conclusions: Phylogroups A, B1, B2, D and VGs (kpsM II, iutA, papA, papC, hlyD, sfaS, focG, afa) were all detected among Ec isolates from healthy humans and patients.
The prevalence of phylogroup A, B2, D and VGs were similar between the healthy human and patient isolates.
Comparing B2 isolates only, iutA, papA, papC and hlyD were significantly more prevalent among patient isolates.
The median no. of VGs/isolate was highest in B2 isolates from patients compared to healthy human isolates.
B2 isolates had more VGs than phylogroup A, B1 and D in both healthy human and patient isolates.

Table 1. Distribution of virulence genes in *Escherichia coli* isolates from healthy humans and patients with UTI

Origin of isolates	Virulence genes							
	kpsMII	*iutA*	*papA*	*papC*	*hlyD*	*sfaS*	*focG*	*afa*
All isolates:								
Healthy humans (n = 109)	55%	37%	19%	17%	10%	7%	6%	4%
Patients with UTI (n = 131)	61%	56%	39%	39%	27%	7%	5%	5%
B2 isolates:								
Healthy humans (n = 39)	97%*	38%	33%	33%	28%	21%	13%	0%
Patients with UTI (n = 62)	76%	63%*	65%*	65%*	56%*	15%	11%	6%

*P < 0.05 between healthy humans and patients.

P1626 Clinical evolution of patients with hepatic abscesses in a period of 6 years (2000–2005)

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Objectives: Pyogenic liver abscesses are normally polimicrobial and caused by enteric bacteria. They can be associated with intestinal or biliary pathology but the 55% of them are cryptogenic.
Methods: Study:retrospective observational. Cases:in-patients diagnosed with hepatic abscess. Information: age, sex, risk factors, associated abdominal diseases, size, location, diagnosis method, isolated microorganism, type of treatment (antibiotics alone versus drainage plus antibiotics), antibiotic treatment duration and complications. Data were analysed with SPSS program for Windows. Stay days at Hospital and antibiotic treatment days were compared between the two groups: Group 1 patients managed only with antibiotic therapy and Group 2: patients managed with percutaneous or surgical drainage and systemic antibiotics. Because of the number of cases and the presence of outliers comparison between stay days and treatment days initially was done by Mann-Whitney test. After that t-test and Levene test were done excluding an outlier patient that had a 158 days of stay.
Results: 60 cases were detected, but only 50 had clinical diagnostic criteria.58% were male. Average age was 70 years old (31–96). Risk factors: 66.7% diabetic, 22% underlying cancer and 32% biliary pathology.38% were cryptogenic. Thirty-seven cases (74%) were discreet abscesses. Most frequent localisation:right hepatic lobe (40%). Microbiologic diagnosis was done by fine needle aspiration (FNA) and blood culture in 17 cases (34%), only by FNA in 17 cases and only by blood culture in 3 cases. Microorganism were not isolated from 13 patients and diagnosis was asserted by clinical, analytical, radiological and evolution criteria. Nineteen cases (38%) were polymicrobial, most frequent microorganisms were: *Escherichia coli*, *viridans* group *Streptococcus*, *Enterococcus* and *Bacteroides* spp. Eighteen cases (36%) were monomicrobial, most frequent microorganisms were: *viridans* group *Streptococcus* and *Escherichia coli*. Complications: 22% had septic shock and attributable mortality was 6.3%. The most frequent empirical treatment was ceftriaxone plus metronidazole (46%) and the second was piperacillin-tazobactam (22%).

	Medical treatment	Drainage + medical treatment	*p*
No. of patients	29	21	
Treatment (days)	34.7±19.2 (14–81)	35.9±19.9 (6–81)	0.688
Stay (days)	20.5±11.6 (3–48)	34.1±31.5 (6–158)	0.013

Conclusions: 1. Patients treated with antibiotics alone had shorter hospital stage, but no differences in antibiotic treatment duration were detected between the two groups. 2. Incidence of liver abscesses of unknown aetiology was lower than described previously in the literature. 3. Incidence of neoplasm as a risk factor was higher in our study.

P1627 A *Trichuris vulpis* parasitosis: an unexpected discovery

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Introduction: *Trichuris vulpis* is an intestinal nematode that belongs to the Trichuroidea superfamily; this worm has a woldwide distribution, and mainly affects dogs, foxes and wolves. This parastosis has been occasionally decribed in veterinary studies. This human disease occasionally is reported affecting in the same way childs and adults. The host becomes infected when it ingests embryonated eggs from the environment.
Clinical case: Eight-year-old girl; Ethiopia native that turned up to her paediatrician for her first health evaluation after adoption. The analytic results were: hemoglobin 10.7 g/dL, hematocrit 34.5%; 9.100 leucocytes/μL; 800 eosinophils/ μL(9%); globular sedimentation speed 35 mm/h; glucose 83 mg/dL; urea 47 mg/dL; albumin 4.9 d/dL, ferritin 8 ng/mL; hide blood in faeces was negative. Feacal samples were obtained for coproparasitological study that was fixed with SAF (saline serum, acetic acid and formol). The Kato method was used to microscope analysis and the concentration by the Ritchie method. This methods demontrated the presence of: *Blastocystis hominis*, *Entamoeba coli*, *Iodamoeba butschlii* and *Giardia intestinalis* as well as yellowish-brown oval-shaped eggs, thick-shelled with two polar plugs, and size 78×40μm. The nematode larvae culture in agar plate was negative. Epidemiological data of living in an africanian country and coexisting with canids, within the microcope analysis of the eggs founded in faeces, estabished the diagnosis of *T. vulpis* infestation. The antihelmintic treatment was mebendazol.
Conclusions: The low T. Vulpis infestation incidence, described by scientific literature, could be underestimated by the scant use of ocular micrometer to measure of eggs size in coproparasitological analysis. The presence of adult worms in faeces must be searched in faeces after the treatment to prove the morfometric data of eggs.

P1628 The gastric involvement of *Orientia tsutsugamushi* in patients with scrub typhus

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Objectives: Scrub typhus is a systemic illness causing generalised vasculitis from *Orientia tsutsugamushi* infection. There are reports of involvement of upper gastrointestinal (GI) tract in scrub typhus. But, the gastric mucosal lesion was influenced by other factors such as pre-existing peptic ulcer and stress due to infection itself. We aimed to prove the presence of *O. tsutsugamushi* in stomach using nested-PCR.
Methods: We selected the patients with scrub typhus undergoing endoscopic gastroduodenoscopy (EGD) in the two university hospitals from January 2003 to November 2007. We evaluated clinical severity index score(CSIS) as the followings: age >56 years old, fever or chill, myalgia or headache, rash, cough, hemoglobin <12 g/dL, total bilirubin

>1.2 mg/dL, albumin <3 g/dL, creatinine >1.3 mg/dL, prothrombin time >14 sec or partial thrombin time >37 sec. The doxycycline was started at the time of diagnosis. EGD was performed in case of patient's agreement. Gastric mucosal biopsies were done using endoscopic biopsy. The CLO test was done to confirm *Helicobacter pylori* infection. Rickettsial DNA was amplified using paraffin-embedded biopsy tissue or fresh tissue by nested PCR.
Results: 35 patients were enrolled during study period. The median age was 62 years old (range, 22 to 81) and males were 13(37.1%). They had no history of peptic ulcer. The median CSIS was 4(range, 2 to 8). Twenty-three patients(65.7%) had GI symptoms including nausea(42.9%), abdominal discomfort (37.1%), dyspepsia(31.4%), vomiting(20%), and diarrhoea(8.6%). EGD findings were gastritis(17.1%), erosion(14.3%), and ulcer(68.6%), which were not related with the symptom duration, CSIS and the presence of GI symptom. Six patients(17.1%) showed positive CLO test in the gastric ulcer. Twnety-two patients (62.9%) were positive nested-PCR for rickettisal DNA. The gastric sample received earlier after therapy tends to be PCR-positive compared with others (2.14±0.71 days vs 4.31±2.10 days, $p < 0.001$). Eighteen patients(51.4%) were positive nested-PCR at the normal gastric mucosa, which was not related with CSIS, GI symptom and EGD finding.
Conclusion: This study showed GI symptoms and gastric inflammation are common. But EGD finding was not related with CSIS and GI symptoms. Gastric inflammation may be related to direct invasion of *O. tsutsugamushi*, which could occur before the symptom develops. The further investigation is needed for pathogenesis of gastric inflammation in scrub typhus.

P1629 Clinical data of *Bartonella* infection in northern Greece

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Objective: Cat scratch disease, caused by *Bartonella henselae*, typically presents with a localised lymphadenopathy. However, there are atypical cases with a wide spectrum of clinical manifestations. The aim of this study was to investigate the epidemiologic and clinical aspects of *Bartonella* infection in northern Greece.
Methods: Betweeen 2000–2006 we examined 1107 patients (522 adults/585 children) suspected of having CSD. The referring doctors were requested to provide the following information: contact with an animal, clinical signs and symptoms, physical findings and the histopathological results in the cases where a lymph node biopsy was performed. All sera were examined using the *Bartonella* IgG/IgM indirect immunofluorescent antibody (IFA) test (Focus kit). A serological diagnosis was made on the basis of either evaluated titers of IgM antibodies >/1:20 or IgG antibodies >/1:256, or the presence of a fourfold rise in the serum IgG titer between acute and convalescent phase. All sera were also tested to *Coxiella burnetti*, *Rickettsia typhi*/*conorii*, *Chlamydia* species, Cytomegalovirus, Epstein Barr virus, Respiratory syncitial virus, *Mycoplasma pneumoniae* and *Legionella pneumophila* to exclude cross reactions.
Results: In 101 patients the diagnosis of *Bartonella* infection was confirmed. 67 patients (29 adults/38 children) appeared typical clinical manifestations of CSD. In 15 out of 29 adults and in 18 out of 38 children contact with cats was reported. In two cases a contact with dogs was also reported. In five patients the histological examination of lymph nodes pointed out histopathological findings compatible with CSD. The most common sites of swollen lymph nodes were the neck (36 cases), followed by the axilla (25 cases), the inguinal region (3 cases) and the submandibular region (3 cases).
In 12 patients with fever of unknown origin (4 adults/8 children), 4 patients (3 adults/1 child) with fever and rash, 4 adults with pneumonia, 3 patients with neurological symptoms, and 5 patients with ocular manifestations the diagnosis of bartonella infection was also established. In six patients with culture negative endocarditis the association of their illness with *Bartonella quintana* infection was strongly suspected, based only on serological criteria
Conclusion: *Bartonella* infection was a rarely notifiable in Northern Greece. Our data indicate, however, that it should be considered a public health problem with several clinical manifestations.

Travel medicine and "exotic" infections

P1630 Travel medicine surveillance in northeastern, Brazil

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Background: Brazil, especially the northeastern coastline region atracts travelers from Brazil and all over the world. Approximately 8% of travelers to the developing world require medical care during or after travel. Due the importance of tourism, travel destination can help physicians select empiric therapy for diseases developing during or after travel.
Methods: We reviewed N=253 patients who were admitted in our infections diseases travel clinic between 02/24/2004 to 12/01/2006 before and after traveling, or during their journey in Recife, Brazil. We conducted a prospective observational monocentric study. Variables available for analysis included gender, age, country, final diagnosis and if medical care was before, during or after travelers. A descriptive statistics of the collected data was done using the software EPI INFO 3.3.2.
Results: 140 (55.3%) were male. The mean age was 33.5 (ranging from one month to 85 years). 141 (55.7%)were Brazilian travelers (n = 303, in transient; n = 30, pre-travel; and n = 3, pos-travel) and 112 (44.3%) were from others countries (n = 35, from Europe; n = 33, from North America). 213 (88.0%) were transient travelers (n = 303 brazilian travelers and n = 310 international travelers). 32 (12.6%) people (n = 30 brazilians) came to the service before travel to counseiling. 14 (5.5%) had been in Malaria and Yellow Fever area less than 30 days before coming to the hospital, and 3 of them (21.4%) came back from their trip with Malaria due to *P. falciparum* (2 from Luanda-Angola; 1 from Manaus-Brazil). Also, 2 (0.8%) people came back from their trip with Viral Meningitis. 22 (8.7%) were assisted at the emergency service due gastroenteritis (acute diarrhoea).
Conclusions: Acute diarrhoea (8.7%) was the principal syndrome during travels and Malaria was the major cause of systemic febrile illness for travelers returning from endemic areas.

P1631 Description of travel characteristics and diagnosis of the main syndromes in travellers attending a tropical medicine unit

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Objectives: To describe and compare the main syndromes and most frequent aetiologies among returning travellers with respect to the geographical area visited, length and type of travel.
Methods: The Tropical Medicine Unit at the Ramon y Cajal Hospital, is a referral unit where immigrants and ill returned travellers are attended since the year 1989
Length of travel was divided into three groups: <30, 30–180 and >180 days. Type of travel was classified into four groups: professional or tourism, high or low risk (PHR; PLR, THR, TLR). Discrete variables were compared by means of Chi squared or Fisher exact test. For quantitative data an independent samples t-test was used.
Results: 2993 travellers were seen at our unit during 1989–2006. A progressive increase in the number of patients was observed over the years. There were 52.1% of men and the mean age was 35 years (interquartile range: 28–40). Long term travel was significantly more frequent in the PHR group (71.5%)
The main reasons for seeking medical advice were: fever: 34.6% (most frequent diagnosis: malaria 25.5%), diarrhoea: 28% (86.2% bacterial gastrointestinal infection), skin diseases: 23% (31.8% skin infections), eosinophilia 19.7% (15.1% filarias) and respiratory tract infections 7.5% (41.3%upper tract infection).

Fever was most frequently diagnosed in travellers returning from Australasia/Oceania (57.1%) followed by South-East Asia (46.2%), diarrhoea: South-West Asia (32.1%) and North Africa (29.5%), eosinophilia: South-East Asia (27.3%), skin disease: South America (31.9%) and South-East Asia (28.7%).

Syndrome	Incidence by type of travel (%)				
	PLR	TLR	THR	PHR	p-value
Febrile	38.9	36.2	40.6	27.5	<0.001
Diarrhoea	26.8	32.1	29.7	24.0	<0.001
Skin disease	22.3	26.3	27.1	17.9	<0.001
Eosinophilia	8.6	6.9	7.4	10.4	0.037

Syndrome	Incidence by length of travel (%)			
	<30 days	30–180 days	>180 days	p-value
Febrile	41.2	29.4	24.6	<0.001
Diarrhoea	30.2	29.5	22.0	0.015
Skin disease	26.3	20.0	18.5	<0.001
Eosinophilia	6.1	9.7	12.5	<0.001

Conclusions: PHR and long term travellers showed less risk of fever, diarrhoea and skin disease on return and an increased risk of eosinophilia. This could be due to the short duration of the former illnesses which typically tend to occur during travel whereas eosinophilia which takes longer to develop is usually detected on return.

P1632 Mycotic aneurysm in northeastern Thailand: the important role of *Burkholderia pseudomallei*

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Objectives: Mycotic aneurysm is a rare condition and related to devastating outcomes. Based on the limited data of the disease, the study was conducted to evaluate the natural history and clinical outcomes of mycotic aneurysm in northeastern Thailand, where melioidosis is an endemic.
Methods: A 15-year retrospective study was conducted in Srinagarind University Hospital during January 1993–June 2007. All patients with mycotic aneurysm based on clinical presentations, radiographic features, intra-operative surgical findings, and pathological reports during the study period were eligible to the study.
Results: There were 37 cases with diagnosis of mycotic aneurysm. The majority of patients were male (29 cases, 78.7%) and elder as the mean (SD) age of 62.9 (12.1) years. Of those, 21 cases (56.8%) had pre-existing diseases, which the 3 most common diseases were diabetes mellitus, chronic kidney diseases and hypertension. All patients presented with pain at the aneurysm site and fever was found in 25 cases (67.6%). Median (range) duration of illness was 14 (3–150) days. Thirty-three cases (89.2%) were involved abdominal aorta. All but one were culture-proven mycotic aneurysm cases. The most common aetiological pathogens were *Burkholderia pseudomallei* (15 cases, 40.5%), followed by *Salmonella* group D (10 cases, 27.0%). *Escherichia coli* and *Pseudomonas* sp. were found two cases each. *Salmonella* group B, *Staphylococcus aureus*, *Aeromonas hydrophila*, *Klebsiella pneumoniae*, *Enterobacter* sp., *Enterococcus* sp., and non-hemolytic *Streptococcus* not group D was found one case each. The most common sites of pathogen isolation were aneurysm (29 cases, 78.4%), followed by blood (7 cases, 18.9%). Overall in-hospital mortality rate was 24.3% (9 cases; 4 cases in *B. pseudomallei* mycotic aneurysm).
There were no statistically significant differences according to age, sex, pre-existing diseases, clinical presentations, site of aneurysm, and mortality between patients with mycotic aneurysm causing by *B. pseudomallei* and other bacteria. Postoperative complications was significantly more common in patients with *B. pseudomallei* mycotic aneurysm compared to those with other bacteria [10 cases (66.7%) vs. 4 cases (19.0%); p = 0.01].
Conclusion: In endemic area of melioidosis, the empirical antimicrobial therapy for suspected mycotic aneurysm should cover *B. pseudomallei*.

P1633 The benign nature of dengue in the elderly in Singapore, 2004

D.C. Lye, V. Lee, Y. Sun, Y.S. Leo (Singapore, SG)

Objectives: Children are at higher risk of dengue haemorrhagic fever (DHF). However, increased mortality in older adults was noted in Cuba and Puerto Rico. In Singapore, patients ⩽55 years comprised 10.1% of dengue cases in 2005 and 22.8% in 2006. This study aims to determine if older dengue patients in Singapore have greater morbidity and mortality.
Methods: All dengue patients admitted to our department in 2004 were retrospectively reviewed. Demographic, clinical, laboratory and outcome data of dengue patients ⩽60 years and <60 years were collected and compared. Using data from entire clinical course, all cases were re-classified into dengue fever and DHF based on World Health Organisation criteria.
Results: Of 1971 laboratory-confirmed dengue cases, 66 were ⩽60 years. Older patients were significantly less likely to be male (44% vs. 66%), and more likely to be Singaporean by nationality (91% vs. 61%), have diabetes (17% vs. 2%), hypertension (48% vs. 4%), ischaemic heart disease (6% vs. 0.1%), hyperlipidaemia (18% vs. 1%) and secondary dengue infections (64% vs. 34%). Clinical features were similar except older patients were less likely to report fever (92% vs. 99%). Older patients were significantly less likely to have leukopenia (32% vs. 51%) and haemoconcentration (0 vs. 5%) on admission. Older patients were not more likely to develop DHF, bleeding, hypotension, severe thrombocytopenia and transaminitis. However, older patients were significantly more likely to receive platelet transfusion (23% vs. 12%). Their length of hospital stay, risk of intensive care unit admission and death were not statistically different.
Conclusions: Despite greater co-morbidity and secondary dengue infection, older dengue patients in Singapore did not have greater morbidity and mortality.

P1634 Lack of benefit of prophylactic platelet transfusion in adult dengue patients

D.C. Lye, V. Lee, Y. Sun, Y.S. Leo (Singapore, SG)

Objectives: Thrombocytopenia is common in dengue and there is concern about risk of bleeding. One retrospective paediatric intensive care study showed no benefit in prophylactic platelet transfusion for thrombocytopenia <30,000/uL. This study aims to determine if prophylactic platelet transfusion has clinical benefit in adult dengue patients.
Methods: All dengue patients admitted to our department in 2004 were retrospectively reviewed. Using data from the entire clinical course, all cases were re-classified into dengue fever and DHF based on World Health Organisation criteria. Patients without bleeding when their platelet count dropped <20,000/uL were evaluated. Prophylactic platelet transfusion was defined as platelet transfusion without clinical bleeding excluding petechiae. Baseline demographic data on admission and clinical data when patients' platelet count first dropped <20,000/uL were compared. Outcome measures included: any bleeding after platelet transfusion, median platelet increase the next day, median time to platelet count ⩽50,000/uL, median length of hospital stay, and death.
Results: Of 1973 laboratory-confirmed dengue patients, 256 developed thrombocytopenia <20,000/uL without bleeding, of whom 188 were given prophylactic platelet transfusion. Baseline demographic, clinical and laboratory features at that platelet threshold were similar, except transfused patients were significantly more likely to be febrile (33% vs.

18%). Median platelet count on day of platelet transfusion was 15,000/uL in transfused vs. 16,000/uL in non-transfused patients. Bleeding occurred subsequently in 1 of 188 transfused (0.5%) and 2 of 68 non-transfused (3%) (p = 0.17). Median platelet increase the next day was 6000/uL vs. 13,000/uL, and median time to platelet ⩽50,000/uL was 3 days vs. 2 days, in transfused vs. non-transfused patients respectively. There was no difference in length of stay and death.
Conclusions: There was no benefit from prophylactic platelet transfusion in adult dengue patients.

P1635 The efficacy of oral ribavirin in children with Crimean-Congo haemorrahgic fever in Iran

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Background: Crimean-Congo haemorrahgic fever (CCHF) is a viral haemorrhagic fever of the Nairovirus group. The disease is endemic in many countries in Africa, Europe and Asia, and during recent years, outbreaks have been recorded in Iran. The data on the efficacy of Ribavirin in in children with CCFH is scanty. This study was conducted to analyse the outcome of this disease, and efficacy of ribavirin therapy in children with CCHF in Southeast of Iran.
Pati ents and method: During 1999 till 2006, 184 confirmed Crimean-Congo haemorrahgic Fever(CCHF) case was admitted in BooAli hospital in Systan province of Iran, of whom 34 cases were below age 18 years old, were included in this study. The diagnosis was confirmed through detection of IgM ELISA and/or genomic segment of PCR CCHF virus. We conducted the outcome analysis of survived treated patients in this study.
Results: Out of 34 children with Crimean-Congo haemorrhagic fever (23 Male, 11 Female)with age range 5 to 18 years old.
24 patients had been treated by oral ribavirin within the initial three days, 8 patients were treated after three days of the onset of disease and two cases had not been treated with ribavirin. Out of the32 treated patients, 6 cases were died. Fatality rate was 18.75% in treated patients. 26 patients with oral ribavirin survived(survival rate=81.25%).
The recovery rate was higher in the children who were treated during the initial 3 days than children who were treated after this time or were not treated (85.2% versus 24.8%).
Both these two children who had not been treated with ribavirin were died.
Conclusion: In children who suffered from CCHF in Iran, treatment with oral Ribavirin can increased survival rate. The recovery rate was higher in the children who were treated during the initial 3 days of the illness. We conclude that the oral Ribavirin is an effective drug in children with CCHF.

P1636 Risk factors for human infection with scrub typhus

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Background: Scrub typhus is a mite-borne infectious disease caused by *Orientia tsutsugamushi*. However, no epidemiological studies have been conducted to examine the risk factors of developing scrub typhus in Korea.
Methods: We conducted this case-control study during the month of May 2006. Cases were 156 patients with scrub typhus who were resided in the southwestern area of Korea (Cholla province and Kwangju city) and registered to the Korean Center for Disease Control and Prevention (KCDC) in 2005. Controls were 130 patient's neighbors who had as same socioeconomic status.
Results: The risk of developing scrub typhus was relatively 2.44 times (95% CI; 1.04–5.69) higher in those who were engaged in farming fruits; 2.05 times (95% CI; 1.09–3.87) higher in those who were engaged in gathering chestnuts; and 3.06 times (95% CI; 1.50–6.22) higher in those who had lunch or a snack on the spot. In addition, it was relatively 0.45 times (95% CI; 0.24–0.83) lower in those who were given informations or a health education programme about the prevention of scrub typhus.
Conclusions: In conclusion, our results indicate that a health education programme will lower the risk of developing scrub typhus in a high-risk group of patients who are engaged in farming fruits, gathering chestnuts and having lunch or a snack on the spot.

P1637 A farmer with a skin ulcer: anthrax or not?

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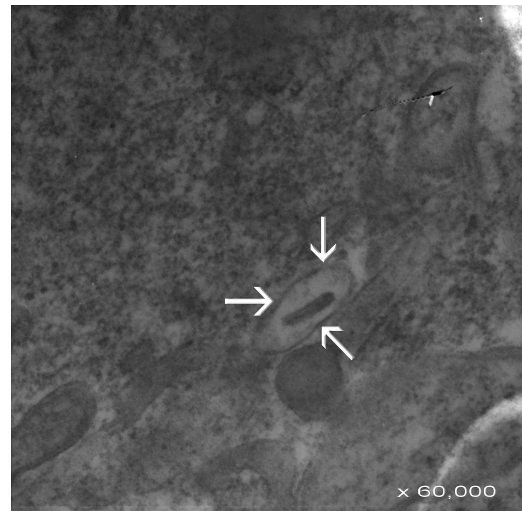
A 52 year-old farmer was admitted to the hospital with a painful ulcer in his left palm. The ulcer was purple in colour with hyperemic and endurated margins. There was hyperaemia and tenderness lining through the left forehand, suggesting lymphangitis or thrombophlebitis. A small painful epitrochlear lymph node was palpable. The patient was afebrile and in good health. Physical examination was unremarkable besides the defined lesion. He had a history of sheep slaughtering in the week before the ulcer had occured. After blood cultures were taken, crystalline penicillin G 3 million units IV q4 h was initiated with the initial diagnosis of cutaneous anthrax and complicated skin ulcer. No microorganism was seen in the Gram staining of the material taken from the edge of the lesion and cultures grew no bacteria. Inflammation and pain resolved at the end of seven days of therapy. A biopsy was performed. Transmission electron microscopy of the biopsy specimen revealed oval virions consisting of a core surrounded by a thin envelope, characteristic for parapoxvirus (figure). The parapoxvirus particles seen were presumed to be orf viruses. Orf virus causes skin diseases in sheep and goats. It is occasionally transmitted to humans. The clinical importance of orf skin ulcer is that it can be misdiagnosed as anthrax, especially in countries where anthrax is still endemic.



P1638 The iliopsoas abscess: aetiology, therapy, and outcome

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Objective: Iliopsoas abscesses are suppurative collections within the fascia surrounding the psoas and iliacus muscles. Psoas abscess is regarded as a rare disease in the medical literature. The incidence of psoas abscess is not known, but it has probably increased in recent years. In this report we describe 13 cases of psoas abscess presenting to a university hospital in Turkey in the last five years.
Methods: We reviewed clinical data about patients diagnosed with a psoas abscess from June 2001 to June 2004 at Baskent University Faculty of Medicine, Adana, Turkey.
Results: The median age of the patients was 47.8 years (range: 18–70 years) with a male to female ratio of 8/5. Out of 13 patients, one had primary psoas abscess, while 12 had secondary psoas abscess. The predisposing conditions were diabetes mellitus, pancreatic carcinoma, autoimmune hepatitis, sickle cell anaemia and chronic renal failure.

Of twelve cases of psoas abscess secondary to vertebral osteomyelitis, five had tuberculosis, one had brucellosis and six had urinary tract infection. The most common complaints were fever and lower back pain. The causative agent was isolated in psoas abscess cultures of 10 cases (*Mycobacterium tuberculosis* in 5 cases, *Brucella melitensis* in 1 case, *Acinetobacter baumannii* in 1 case, meticillin sensitive *Staphylococcus aureus* in 2 cases and meticillin resistant *S. aureus* in 1 case). Culture results were negative in two patients and it was not possible to obtain specimen from one patient. Eleven patients underwent percutaneous drainage, which was successful in all cases. Two patients were treated with surgical drainage.
Conclusion: With the advent of modern imaging techniques, the diagnosis of psoas abscess without typical clinical symptoms has become easier. However, despite the improvement in diagnostic techniques, this disease remains a problem as the development of abscesses is often associated with an underlying chronic illness. It is striking that in our series none of the patients had psoas abscess secondary to a disease of the digestive tract, while this is the most common cause of secondary psoas abscess in the literature. Even if the incidence of tuberculosis is actually increasing, tuberculosis abscesses are rare in Western countries. However, it should be kept in mind that Brucellosis and Tuberculosis can be the cause of psoas abscess in developing countries like Turkey where tuberculosis and brucellosis are still endemic.

Food and waterborne pathogens and infections

P1639 *Salmonella typhimurium* and norovirus food-borne outbreak in a restaurant

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Objectives: *Salmonella* Typhimurium (ST) and norovirus (NV) are the leading cause of food borne outbreaks of gastroenteritis in Catalonia; however outbreaks caused with mixed viral-bacterial gastroenteritis are unusual. This report describes the first food borne detected in Catalonia with ST and NV together implicated. Two groups of costumers ate on the same restaurant in a two consecutive days before became ill.
Methods: A study of historic cohorts was performed. The case was defined by the presence of diarrhoea and vomiting. Data were gathered on age, sex, foods consumed and clinical symptoms. Attack rates and relative risk were calculated. Food and stool samples from cases and food handlers were analysed for bacterial and viruses (test RT-PCR). Bacterial isolated strains were studied using pulsed-field gel electrophoresis (PFGE). Food handlers were asked about food procedures and a inspection of the restaurant was undertaken.
Results: The outbreak occurred from August 7 to 9, 2005 involving 20 cases (65% total attack rate). Clinical symptoms included general malaise (95%), diarrhoea 85, headache (85%), abdominal pain (80%), muscular pain (80%), fever (75%), nausea (33%) and vomiting (25%). Median duration of the disease was 4 days.
There were a significant association with the disease and eating mixed vegetables (RR = 4.95; CI 95%, 1.40–17.50) and fish salad (RR = 2.25; CI 95%, 1.25–4.05). Stool samples of 4 cases were ST positives ST, one case for NV and 5 cases for ST and NV. Stool samples from food handler were ST positives (1) and both ST and NV (2). Furthermore ST was also isolated in the food samples. The ST strains from 9 confirmed cases, from food handlers and food had identical PFGE patterns. Restaurant inspection detected that cross contamination of food were possible.
Conclusion: This food borne epidemic outbreak was caused by ST and NV. Epidemiological analysis shows that mixed vegetables and fish salad were the main foods implicated. Microbiological information is consistent with the outbreak could occurred as a result of food contamination by a food handler. ST infection could be also as a result of cross contamination and mishandling of food.

P1640 Clinical features and possible sources of sporadic *Yersinia enterocolitica* infections in Finland

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Objectives: *Yersinia enterocolitica* is an important cause of gastroenteritis in Finland causing around 500 infections yearly. *Y. enterocolitica* species is divided into several bio- and serotypes. The ability to cause gastrointestinal symptoms differ between the bioserotypes as well as the possible sources of infection. We conducted a case-control study to characterize the clinical features and to identify sources of *Y. enterocolitica* bioserotypes.
Methods: All *Yersinia* strains isolated in 10 clinical microbiology laboratories in 2006 were collected. Patients with culture confirmed *Y. enterocolitica* infection were enrolled to questionnaire study. Controls matched on age-, gender- and geography were randomly selected from population registry. Questionnaire included questions related to *Yersinia* infection, consumption of different food items, traveling etc.
Results: A total of 294 cases were accepted to case control study. Most patients had biotype (BT) 1A (N=187) finding, bio-/serotypes 3–4/O:3 or 2/O:9 were detected in 61patients. Patients with other serotypes were excluded from further analyses. Patients with 3–4/O:3 or 2/O:9 findings were younger than those with BT 1A findings ($P < 0.001$ for difference in means) and small children under three years were overrepresented among those with serotypes O:3 or O:9 (10/61=16%). Many patients with biotype 1A were unable to define any special symptomatic period. Diarrhoea and abdominal cramps were most common symptoms, fever was more common among patients with serotypes O:3 or O:9 and vomiting among subjects with biotype 1A. Significant associations in preliminary analyses were found between both 1A and O:3 or O:9 types and travelling abroad. Serotypes O:3 or O:9 were associated to eating or tasting medium or raw pork. Those who always washed vegetables had decreased risk of *Y. enterocolitica* infection.
Conclusion: Clinical feature of *Y. enterocolitica* infections differ between bio-serotypes significantly. Substantial proportion of *Y. enterocolitica* infections in Finland is associated with traveling. Pork is a significant source of serotype O:3 or O:9 *Y. enterocolitica*, but it doesn't explain all infections.

P1641 Severity of non-typhoid salmonellosis as a predictor of 0–30 and 31–365 day mortality

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Objectives: To evaluate whether hospitalisation, ordering of blood cultures, or detection of bacteraemia were predictors of mortality in patients with non-typhoid salmonellosis (NTS).
Methods: Population-based registry study comprising all patients with a first-time NTS detected in stool cultures, in North Jutland County (Denmark) from 1995 through 2003. Patients were categorised into four groups: group 1) not hospitalised; group 2) hospitalised 30 days within their NTS without blood cultures (BC) being ordered; group 3) hospitalised with negative BC only; group 4) hospitalised with bacteraemia. We obtained data in the county's hospital discharge registry on comorbidity as recorded from 1977 and hospitalisation. Complete follow-up was possible through the Danish civil registration system. Age and comorbidity adjusted Cox's regression analyses were used to compute mortality rate ratios (MRR) with 95% confidence intervals (CI) at 0–30 and 31–365 days.
Results: Among 1,764 NTS patients, 1,082 (61.3%) were not hospitalised, 344 (19.5%) were hospitalised without BC being ordered, 245 (13.9%) were hospitalised with negative BC, and 93 (5.3%) had bacteraemia (*Salmonella* in 81, other pathogens in 12). Within one year, 2 (0.2%) died in group 1, 14 (4.1%) in group 2, 20 (8.2%) in group 3, and 19 (20.4%) in group 4. Using group 2 as reference, 0–30 day adjusted MRR (95% CI) could not be calculated in group 1 (no deaths), whereas they were 1.8 (0.6–5.7) and 1.3 (0.4–4.6) in groups 3 and 4, respectively. For 31–365 days, adjusted MRR (95% CI) were 0.16

(0.03–0.78) in group 1, 1.6 (0.69–3.9) in group 3, and 2.3 (1.0–5.5) in group 4.

Conclusion: The general practitioner's decision to hospitalize NTS patients was a predictor of short-term and long-term mortality independent of age and comorbidity. When hospitalised, the physician's decision to obtain blood cultures or the detection of bacteraemia were independent predictors of mortality, albeit the statistical precision was low.

P1642 Prosthetic mitral valve endocarditis due to *Salmonella paratyphi* B

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Objectives: Endocarditis due to *Salmonella* species is a rare clinical entity. We report a case of prosthetic mitral valve endocarditis caused by *Salmonella paratyphi* B in which vegetation could be demonstrated by transoesophageal echocardiography.

Case: A 37-year-old male patient was admitted to the hospital in September 2007 with complaints of fever, cough, expectoration, palpitation and generalised aches for two weeks. He had a history of mitral valve replacement ten years ago. Physical examination revealed tachycardia, jaundice, hepatomegaly, crepitation on both sides of the chest and metallic sound of prosthetic valve. The leukocyte count was 4000/mm^3, hemoglobin 15.4 g/dl, platelets 39000/mm^3, erythrocyte sedimentation rate 49 mm/hour. In urinalysis leukocytes (160/mm^3) were detected. Chest X-ray showed bilateral lung infiltrates. Transthoracic echocardiography did not reveal any vegetation. The treatment started with ampicillin-sulbactam (4×1.5 g) and ciprofloxacin (2×400 mg) intravenously against pneumonia and urinary tract infection, but urine culture was negative. All of six blood cultures yielded *Salmonella paratyphi* B sensitive to ampicillin, ciprofloxacin, chloramphenicol, cefotaxime and trimethoprim-sulfamethoxazole. None of stool cultures revealed salmonella. On day 8, his clinical status deteriorated; dyspnea, arrhythmia, edema, leukocytosis (greater than 20000/mm^3) were added. No vegetation was detected by repeated transthoracic echocardiography. Medications for cardiac failure were added to the treatment. On day 10, transoesophageal echocardiography showed two vegetations (1.7×0.4 cm and 0.3×0.3 cm) on prosthetic mitral valve. Cardiovascular surgeons did not suggest surgical intervention. The patient had transferred to a cardiology clinic on day 11 because of the worsened clinical status. We learned that the antimicrobial treatment was continued in the cardiology clinic and that mental functions of the patient worsened because of temporal infarction detected by cranial tomography. After eigth weeks of the treatment he was discharged from hospital with a sequel without any surgical intervention.

Conclusion: Complication and mortality rate of salmonella endocarditis is high and early surgical intervention is needed commonly. In this case we could make the diagnosis of endocarditis on 10th day of admission. We strongly recommend early practice of transoesophageal echocardiography in patients with suspected endocarditis.

P1643 Significant presence of adenoviruses in raw and treated sewage derived from biological treatment plants in Greece

G. Komninou, D. Venieri, G. Diamandi, E. Jelastopulu (Patras, GR)

Objectives: Water quality and, therefore human health may be significantly affected by the presence of pathogenic enteric microorganisms, derived from sewage discharged to the aquatic environment. Outbreaks of enteric virus disease have been linked to water at various times and to different causes. Adenoviruses are pathogenic to humans and their presence in the environmental samples (polluted waters) may cause infections. Adenoviruses are the only human enteric viruses that contain DNA. Many adenoviruses serotypes are difficult to culture in regular cell lines. The stability of human adenoviruses in environmental waters and their resistance to physicochemical treatment processes used by sewage treatment plants may facilitate their transmission.
Our purpose was to perform a complete procedure for virus concentration from sewage and the detection of the specific viruses by amplification of DNA and cDNA with the appropriate primers, in order to overcome the problems of the cultivation methods concerning adenoviruses.

Methods: In this study raw and treated sewage samples were collected from three treatment plants in Greece. Two of them are located in the prefecture of Achaia (Patras and Rio) and the other in the prefecture of Preveza (city of Preveza).
In total, 60 sewage samples (30 raw and 30 treated sewage samples) were collected, during the period March 2006–June 2006.
Our approach consisted of a simple concentration of viruses from sewage samples followed by nested PCR in order to increase the sensitivity of adenovirus detection.

Results: In total, adenoviruses were detected in 55% of the samples tested. Reviewing results obtained from each treatment plant separately, positive samples from Patras, Rio and Preveza were 75%, 67% and 50%, respectively. Furthermore, adenoviruses were detected in 24 raw sewage samples (80%) and in 9 treated sewage samples (30%).

Conclusions: The detection of adenoviruses, in raw and treated sewage with this specific, fast and sensitive method is a valuable indicator of quality control of the process applied by the biological treatment plant, as it can be used for the examination of treated effluents as well as raw sewage samples. The significant isolation of adenoviruses in treated sewage indicates their stability as virological indicators of the pollution of the environment and their more persistence in sewage.

P1644 Detection of enteroviruses in raw and treated sewage in Greece

D. Venieri, G. Komninou, K. Nelly, E. Jelastopulu (Patras, GR)

Objectives: Enteroviruses have been associated with outbreaks of waterborne non-bacterial gastroenteritis and are of important concern for public health. Significant numbers of viruses can be isolated from faeces and urine of humans as well as from sewage and polluted waters. The purpose of this study was to investigate the presence of enteroviruses in raw and treated sewage samples derived from a biological treatment plant in Greece, applying molecular biological techniques. Furthermore, there was an effort to overview enteroviruses' inactivation, as a consequence of the disinfection of sewage.

Methods: In this study raw and treated sewage samples were collected from three treatment plants in Greece. Two of them are located in the prefecture of Achaia (Patras and Rio) and the other in the prefecture of Preveza (city of Preveza).
In total, 60 sewage samples (30 raw and 30 treated sewage samples) were collected, during the period March 2006–June 2006. Our approach was consisted of a simple concentration of viruses from sewage samples, followed by RT-nested PCR in order to increase the sensitivity of enterovirus detection.

Results: In total, enteroviruses were detected in 8% of the samples tested. All samples from Patras' treatment plant were negative, while in Rio and Preveza positive samples for the presence of enteroviruses were 8% and 9%, respectively. What seems to be important is the detection and isolation of enteroviruses in raw and treated samples, as well. Specifically, 13% of raw sewage samples were positive for the presence of enteroviruses, while this proportion for treated sewage samples was lower and 3%.

Conclusions: The detection of enteroviruses, in raw and treated sewage with this specific, fast and sensitive method is a valuable indicator of quality control of the process made by the biological treatment plant. This methodology can be easily used for the examination of treated effluents and raw sewage samples, for the fulfilment of epidemiological studies as well as for the implementation of monitoring and management plans.

P1645 The survey of common bacterial contamination in bottled mineral water

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Background: Bottled mineral water, generally considered more pure than tap water in developing countries. Immunocompromised patient and whom with commorbid condition often receive bottled mineral water under the assumption that it is safer than tap water. To determine the risk of common bacterial contamination of commercial bottled mineral water in Iran, we conducted this study in 2006.
Materials and Methods: The design of the research is descriptive study. 68 samples (35 brand named company) of bottled mineral water was collected from different province of Iran. Initially 200 ml of bottled water was filtered with 0.45 micrometer pore (Sartorius label). The filter was divided by sterile pence and scissor to multiple pieces, suspension in nutrient broth and centrifuged. The culture was made by use of selective media: nutrient agar and broth, dextrose sodium azide agar, yersinia agar, and *Clostridium* selective agar. The bacterial contamination were detected after incubation in 42ºC, 35ºC and 25ºC for 24 h, 48 h and 7 days.
Results: Out of 68 samples of bottled mineral water (35 brands), 41 samples (60%) showed evidence of contamination with common bacterial, including 15 samples (36%) Gram-positive sporforming bacilli, 20 Samples (49%) Gram-positive non sporforming bacilli (diphteroids) and 6 Samples (15%) Gram-positive cocci. The marker organisms, i.e. the classic faecal contamination indicators were not detected in any of samples.
Conclusion: This finding indicated that the use of bottled mineral water in immunocompotent persons is safe, but the use of this bottled mineral water in immunocompromised patients should be done with meticulouse precaution. Some of bacteria were known human commensals suggesting contamination of waters prior to bottling. It is recommended that bottling, packaging and distribution of this water should be done more surveillance.

P1646 *Vibrio cholerae* O139 requires neither capsule nor lipopolysaccharide O side chain to grow inside *Acanthamoeba castellanii*

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Objectives: *Vibrio cholerae* O139, the causative agent of cholera, has grown and survived in the aquatic free-living amoeba *Acanthamoeba castellanii*. The aim of the present study was to examine the ability of the clinical isolate *V. cholerae* O139 MO10 to grow with each of *A. castellanii*, *A. culbertsoni* and *A. polyphaga* as well as to determine effect of bacterial capsule and LPS O side chain on the intracellular growth of *V. cholerae* O139 MO10 in *A. castellanii*.
Methods: *Acanthamoeba* species and *V. cholerae* strains were alone and co-cultivated for two weeks. Gentamicin assay was used to kill extracellular *V. cholerae* as well as to examine ability of amoeba to protect intracellular *V. cholerae*. Interaction between microorganisms was studied by viable count, mannose assay, electron microscopy and statistical analysis.
Results: The results showed that *A. castellanii*, *A. culbertsoni* and *A. polyphaga* grew in the presence of *V. cholerae* O139 MO10 and the amoeba number increased ten-fold, 2 times and 4 times after 14 days, respectively.
Growth of the wildtype *V. cholerae* MO10, *V. cholerae* MO10-T4 lacking capsule, and *V. cholerae* Bengal-2R lacking LPS O side chain was enhanced significantly in the presence of *A. castellanii* ($p = 0.00003$). Intracellular growth of *V. cholerae* strains in *A. castellanii* was not significant; p value of Chi-2 test was 0.999).
The addition of mannose did not affect intracellular growth of *V. cholerae* MO10 in *A. castellanii*, which indicated that the mannose did not inhibit adherence of *V. cholerae* to *A. castellanii* explaining that *V. cholerae* cells adhered nonspecifically to *A. castellanii* and nonspecific adherence plays a role of bacteria attachment to *A. castellanii*. The number of viable *V. cholerae* O139 MO10 inside *A. castellanii* with or without mannose was not significantly different ($p = 0.94$).
Electron microscopy showed that the intracellular localisation of the bacteria was in vacuoles in the cytoplasm of trophozoites a few hours after co-cultivation. Multiplication of bacterial cells occurred in the cytoplasm of trophozoites one day after co-cultivation and the bacteria were found in the cysts of *A. castellanii* 6 and 7 days after co-cultivation.
Conclusions: Neither the capsule nor the LPS O side chain of *V. cholerae* O139 was found to play an important antiphagocytic role against *A. castellanii* disclosing the intracellular behaviour of *V. cholerae* as well as the role of Acanthamoebae as environmental hosts for *V. cholerae*

P1647 Ability of *Acanthamoeba polyphaga* to host cholera toxin producing *Vibrio cholerae* O1 classical strains

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Objectives: *Vibrio cholerae* O1 is a waterborne bacterium that infects humans and causes cholera. Since the infections dose is 10^8-10^9 cells, *V. cholerae* requires a host for multiplication to be able to reach this dose. *Acanthamoeba* is a genus of free-living amoeba that can host pathogenic bacteria and acts as a model cell to study interaction between eukaryotes and prokaryotes. Our previous studies have shown that *V. cholerae* O1 and O139 can grow and survive symbiotically inside *Acanthamoeba castellanii*, which indicates that free-living amoeba may have a roll in the epidemiology of *V. cholerae*. The aim of this study is to examine ability of *A. polyphaga* to host *V. cholerae* O1 classical.
Methods: *A. polyphaga* and *V. cholerae* were alone and co-cultivated for two weeks. Gentamicin assay used to discriminate between extracellular and intracellular growing *V. cholerae*. Interaction between amoebae and bacteria was studied by viable count, electron microscopy and statistical analysis.
Results: Number of viable *A. polyphaga* in absence or presence of *V. cholerae* increased from 2×10^5 cell/ml at day 0 to 1.6×10^6 cell/ml and to7×10^5 cell/ml at day 15, respectively. Presence of *V. cholerae* did not inhibit growth of the amoebae; p of t-test was 0.30.
Viable count of *V. cholerae* in presence of *A. polyphaga* was 2×10^6 CFU/ml at day 0 and increased to 2×10^9 CFU/ml at day 1. The bacteria survived more than two weeks and the viable count was 2×10^8 CFU/ml at day 15. Viable count of *V. cholerae* in absence of *A. polyphaga* decreased from 2×10^6 CFU/ml at day 0 to 0 CFU/ml. The difference in growth was significant ($p < 0.0.5$).
V. cholerae grew inside *A. polyphaga* from 0 CFU/l at day 0 to 2×10^6 CFU/ml at day 1. The intracellular bacteria survived more than two weeks and their viable count was 5×10^5 CFU/ml at day 15. Electron microscopy visualised the intracellular localisation of *V. cholerae* cells in vacuoles of the trophozoites as well as in cysts of *A. polyphaga*.
Conclusions: *A. polyphaga* and *V. cholerae* live together in co-culture. Growth of the amoebae was not inhibited, and growth of the bacteria was enhanced and the bacterial growth occurred inside amoeba cells indicating a symbiotic relation between *A. polyphaga* and *V. cholerae* O1 classical strains.

P1648 An investigation into clostridia toxins as a cause of gastro-intestinal disease in companion animals

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Objectives: Diarrhoeal disease is a common clinical presentation in companion animals. The most common bacterial agents are *Salmonella* species, and *Campylobacter* species. Studies have clearly demonstrated that *Clostridium* species can cause a number of disease states in production animals; however, evidence of investigation into *Clostridium* species as a causative agent of gastro-intestinal disease in companion animals is scant. It would be expected to isolate *Clostridium* species from most clinical faecal samples, however, it is important to demonstrate toxogenicity in these isolates, as toxin production is the primary pathogenicity factor. Two species of *Clostridium* have been primarily

connected to intestinal disease in both humans and animals; these are *C. perfringens* and *C. difficile*. The primary aim of this study is to identify the presence of *Clostridium* species in the faecal samples of companion animals with no other detectable disease process to explain their gastro-intestinal symptoms. The investigation will include culturing the samples for the confirmation of *Clostridium* species and toxin detection for the presence of *C. perfringens* enterotoxin and *C. difficile* toxins A and B.

Methods: An investigation was carried out into the prevalence of *C. perfringens* and *C. difficile* in companion animals. We investigated faecal samples from 132 dogs and 56 cats, all with a history of diarrhoea. The investigation consisted of testing all samples (in two groups of 94) using the ELISA tests, *C. difficile* TOX A/B II and *C. perfringens* enterotoxin. The first batch of samples were cultured anaerobically and pheynotypically identified as *Clostridium* species. The second set of samples were further sub-cultured to achieve a pure growth and identified using *Clostridium* PET.

Results: 27.3% of dogs and 14.3% of cats were positive using the *C. difficile* ELISA. 49.2% of dogs and 51.8% of cats were positive on the *C. perfringens* ELISA. Culture results showed the first set of specimens to be 51.6% positive for Clostridial growth in dogs and 50% in cats. The second set showed that 2.9% of dogs and 3.8% of cats carried *C. difficile*, 2.9% of dogs and 7.7% of cats carried *C. perfringens* and 42.6% of dogs and 46.2% of cats carried other species or unidentified *Clostridium* species.

Conclusion: This investigation demonstrates that dogs and cats carry both *C. difficile* and *C. perfringens*. This study used samples from animals that had confirmed cases of diarrhoea. Further work needs to be carried out to investigate the prevalence of *Clostridium* species in healthy animals and to identify whether it is a significant causative agent of diarrhoea or a harmless commensal present in the majority of animals.

P1649 Farm to fork tracing of broiler-derived salmonellae and thermotolerant campylobacters in a Hungarian county using molecular epidemiology methods

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Objectives: Our aim was to follow salmonellae and campylobacters from flock through abattoir to retail shops in seven broiler flocks and to monitor human disease occurring in the region where the meat was sold. Five flocks were kept consecutively in the same henhouse.

Methods: Faecal samples were collected at least at three weeks of age and before slaughter. At abattoir hygienic, neckskin and breast filet, while at shops different meat samples were collected. Sampling followed the official guidelines. All human stool samples sent for examination from patients older than one year from the region were included. Cultured salmonellae and campylobacters were subjected to phage- and fla-typing, respectively, results were confirmed by PFGE.

Results: Only the two independent flocks were *Salmonella* positive at premise. Neckskin samples were always positive, but prevalence in faeces-negative flocks was lower. Abattoir meat of negative flocks yielded only one of five or no positive samples, while in case of positive flocks was always 100% positive similarly to retail meat. Retail meat of only one abattoir-negative flock remained negative in the shop indicating that meat may become contaminated in the shop. All 109 isolated salmonellae were S. Infantis, and together with all 17 human S. Infantis isolates belonged to the clone known to be widespread in Hungary.

All flocks were *Campylobacter* positive at three weeks and before slaughter, but generally negative at earlier ages. Contamination has in all flocks been passed to abattoir- and retail meat (30/35 and 45/55 positives, respectively). *C. jejuni* was predominant. Culture-confirmed campylobacteriosis occurred in 2.5–4.5% of sampled enteritic patients, showing an incidence of 3.1 to 8.3/100000 per month.

Typing of campylobacters revealed that all flocks harboured a unique strain detected in the majority of animal-derived, abattoir and retail meat samples. Passing of campylobacters between flocks was rare even in flocks kept in the same henhouse. Human isolates were diverse, all flock-specific patterns were detected in human samples.

Conclusion: This work models a way to trace food-borne pathogens from farm to fork. S. Infantis was highly clonal, widespread among flocks and human cases. Campylobacters, in contrast, show significant diversity, and each broiler flock seems to have a unique dominant *Campylobacter* clone, which led to a high diversity in human disease.

The work was supported by GVOP-3.1.1.-2004–05–0472/03 grant.

P1650 Comparison of the microbial load of incoming and distal outlet water from dental unit water systems

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Objectives: The objective is to compare the total microbial load of the incoming and distal outlet water in dental unit water systems using two methods, traditional culture method and epifluorescence microscopy. Additionally, the presence of *Legionella pneumophila* bacterium which is responsible for Legionnaires' Disease was also tested by standard ISO method in distal outlet waters.

Method: 123 water samples were taken from the high-speed handpieces lines, air-water syringe lines and source (incoming) water supplies from 41 dental units. 21 dental units were randomly selected from private practices, whereas the other 20 units were at the same hospital's dental clinic. The samples were assayed for live/dead bacteria (DAPI+CTC dual dye combination), heterotrophic bacterial counts and presence of *L. pneumophila* bacteria (*L. pneumophila* only in high-speed handpiece outlet water).

Results: In our study, 39 out of 41 dental units were not able to meet the limit of 200 CFU/ml in dental unit water. Live bacterial counts were found 1–1.5 orders of magnitude higher than aerobic mesophilic heterotrophic bacteria. *L. pneumophila* (serogroup 2–14) was isolated from 5 units out of 41. Source water of eight unit was heavily contaminated which were fed up by commercially bottled drinking water.

Sample source	N	Mean count $\log_{10}$ CFU/ml
AMHB		
Inlet water	41	3.17
High-speed handpiece	41	3.51
A/W Syringe	41	3.29
Live Microorganisms		
Inlet water	41	4.02
High-speed handpiece	41	4.09
A/W Syringe	41	4.83
Total Microorganisms		
Inlet water	41	8.95
High-speed handpiece	41	9.41
A/W Syringe	41	9.03

AMHB: aerobic mesophilic heterotrophic bacteria; A/W: Air/water.

Conclusion: In general, the microbiological quality of dental unit water from dental practice is poor. In some practices the source water was found heavily contaminated, but the use of water with an initial low contamination level did not prevent its increase within dental units. Dental unit waterlines must be maintained regularly to deliver output water of an acceptable microbiologic quality.

MDR Gram-negatives – clinical epidemiology

P1651 *Klebsiella pneumoniae* ESBL, a protracted outbreak

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Objectives: Increasing numbers of multi-resistant *K. pneumoniae* isolates were found during May-Aug 2005 in Uppsala University Hospital, predominantly in urinary samples. The objective of the study was to identify risk factors for colonisation, develop screening strategies, and to reduce spread of resistant Enterobacteriaceae.

Methods: Isolates were typed with PhenePlate and PFGE, and belonged to a single clone, producing CTXM-15. Numbers of new cases were few after November 2005, but increased greatly during June-August 2006. The hospital then formed a *Klebsiella* ESBL steering group and working group with representatives from Microbiology, Infectious disease, Infection control, and Hospital administration.

Interventions used to terminate the outbreak:

– Placing cases with urinary catheters, diarrhoea, and/or discharging wounds in single rooms
– Enforced contact precautions for all levels of staff: short-sleeved working clothes, alcohol hand disinfection before and after any patient contact, gloves and plastic aprons at risk of soilage.
– Emphasis on hand hygiene for patients and visitors.
– Antimicrobial stewardship

To improve case-finding, screening strategies were developed, concentrating on stool samples from all patients in all wards, using selective media and PCR for CTXM group 1.

Results: Over 40 000 screening samples were taken Oct 2006-Aug 2007. From May 2005 to September 2007, 257 patients were found to carry the outbreak strain in >50 different wards and departments, but not in intensive care units. A case-control study identified naso-gastric feeding, urinary tract catheters, diarrhoea, surgery, and use of third-generation cephalosporins/fluoroquinolons/ carbapenems as independent risk factors.

During June-Aug 2007, only 4 new clinical cases occured, as compared to 41 during June-Aug 2006. Use of hand alcohol increased from 31 to 81 ml/ per patient day. Improved compliance with contact precautions was observed. Cephalosporin use was reduced from approx 12000 to 4000 DDD/ six months. New patient beds were opened and staffing increased, to reduce overcrowding in risk wards. The cost of the outbreak is estimated at 3 million EUR.

Conclusion: When cases do not seem to be related in time and space, and most patients are only colonised, an outbreak can become very large before it is recognised. Joint efforts are needed to control such an outbreak, focussing on hand hygiene for patients and staff, reduction of overcrowding, and antimicrobial stewardship.

P1652 Antimicrobial resistance and extended-spectrum β-lactamase production of Enterobacteriaceae from inpatients and outpatients with urinary tract infection

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Objectives: Extended-spectrum β-lactamases (ESBL) are important resistance traits for nosocomial isolates Enterobacteriaceae and their spread in the community generates increasing concern. This study was performed to investigate the prevalence of ESBL producers in Enterobacteriaceae isolated from both inpatients and outpatients with urinary tract infection and their resistance to antimicrobial agents.

Methods: A total of 965 strain of Enterobacteriaceae were isolated from urine samples; 434 isolates from inpatients and, 531 from outpatients from June 2006 to September 2007. Suspected strains are presumptively defined as ESBL producers according to result of disc diffusion method, using ESBL marker antibiotics ceftazidime (CAZ), cefotaxime (CTX) and aztreonam (ATM). Those isolates were retested with double-disc sinergy test (DDST)-CAZ, CTX, ATM and amoxicillin-clavulanic acid (AMC) disc implementation (CLSI). Enhancement of inhibition zone (or so called ghost zone) indicated presence of ESBL. Antimicrobial susceptibility to β-lactam antibiotics, gentamicin, trimethoprim-sulfamethoxazole, ciprofloxacin, nitrofurantoin was performed by disc diffusion method according to the recommendations of the CLSI.

Results: The species distribution as follows: *Escherichia coli* 79.3%, *Klebsiella* spp. 12.7%, Entrobacter spp. 3.2%, *Proteus* spp. 3.0%, *Citrobacter* spp. 1.0%, *Morganella morganii* 0.4%, *Serratia marcescens* 0.3%, Pantoea agglomerans 0.1%. The average prevalence of ESBL producers was 47.0% among isolates from inpatients and 8.7% among outpatient isolates. Approximately 95% of the ESBL-producing strains belonged to just two species *E. coli* (n = 194/25.4%) and *Klebsiella* spp. (n = 45/36.9%).

The resistance rates of non-ESBL producers and ESBL producers to imipenem 0.0% versus 1.6%, to nitrofurantoin 5.7% versus 30.5% (p = 0.000), to gentamicin 15.3% versus 63.6% (p = 0.000), to ciprofloxacin 25.8% versus 58.0% (p = 0.000), to trimethoprim-sulfamethoxazole 38.2% versus 68.8% (p = 0.000) respectively.

Conclusion: ESBL production has been obsorved in large percantage of urinary isolates and their expression is usually associated with multi-drug resistance. Thus, surveillance of ESBL dissemination appears to be important also community acquired infections.

P1653 Risk factors and clinical outcome for extended-spectrum β-lactamase producing *Escherichia coli* blood stream infection

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Objective: Extended-spectrum β-lactamase (ESBL)-producing *E. coli* have been increasingly isolated around the world. The aim of this study is to determine the risk factors and the influence on clinical outcome of ESBL-producing *E. coli* bloodstream infection (BSI). We also investigated the influence of quinolone-resistant *E. coli* BSI on the clinical outcome.

Methods: A retrospective study of 30 patients with bacteraemia due to ESBL-producing *E. coli* and of 107 patients with non-ESBL-producing *E. coli* in a tertiary hospital from 2005 to 2007 was conducted upon review of patients' medical records. 107 patients with non-ESBL-producing *E. coli* BSI were divided into two groups according to the resistance to quinolone and compared with each other. All data were analysed by Statystical Analysis System (version 9.13).

Results: Multiple logistic regression analysis identified the prior administration of 3rd generation cephalosporins [odds ratio(OR) = 11.177, p = 0.022], history of hospitalisation at intensive care unit (OR = 20.917, p = 0.0026) in the previous 30 days were the independent risk factors for ESBL-producing *E. coli* BSI. Patients with bacteraemia due to ESBL-producing *E. coli* were treated with appropriate antibiotics less often than controls (OR = 9.83, p = 0.0054). And clinical success was slightly more frequent in the group with ESBL-producing *E. coli* BSI than control group (OR = 20.815, p = 0.0031). The status of ESBL production showed no association with hospital mortality (OR = 3.23, p = 0.2648). Among 107 patients with non-ESBL-producing *E. coli* BSI, 43%(47/107) of isolates were resistant to quinolones. Patients with bacteraemia due to quinolone resistant *E. coli* were treated with appropriate antibiotics less often than control group (OR = 418.43, p < 0.0001). But, there was no significant difference in clinical success (OR = 1.036, p = 0.9676) and hospital mortality (OR = 2.859, p = 0.3448).

Conclusion: The administration of 3rd generation cephalosporins, history of hospitalisation at intensive care unit in the previous 30days were the independent risk factors for ESBL-producing *E. coli* BSI. And the status of ESBL production showed no association with hospital mortality though patients with bacteraemia due to ESBL-producing *E. coli* were treated with inappropriate antibiotics more often.

P1654 Multidrug-resistant *Pseudomonas aeruginosa* among hospitalised patients: risk and prognosis factors

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Objectives: Due to the increasing strains of multidrug resistance *P. aeruginosa* (MRPA) we want to know the risk and prognosis factors associated with its acquisition.
Methods: A case-control study was designed based in patients with isolated nosocomial MRPA. The identification and susceptibility test of the strains was performed using the system Walk-Away® (Dade-Behring) and E-test® (Izasa) methods according to CLSI standards. Statistical analysis was performed with the SPSS/PC version 11.5.
Results: From October 2002 to December 2004 were isolated 81 nosocomial strains of MRPA that were matched by age and sex with other 81 nosocomial strains of susceptible *P. aeruginosa* (SPA). Most of them were male (77%) and the average age was 61.73 years. The majority of patients with MRPA stayed in ICUs (54.3%, $p < 0007$). The average stay from admission to the isolation (27.7 $p < 0043$), as well as length of hospitalisation after that (28.69, $p < 0001$) were longer in the cases. 57.7% of MRPA were associated with the development of severe infection ($p < 0016$). Both the crude mortality (30.9% vs. 23.5%) and the attributable (12.3% vs. 2.5%, $p < 0.016$) was greater in the cases. All invasive procedures were more common in patients with PAMR, being statistically significant tracheal intubation, mechanical ventilation ⩾48 hours, nasogastric tube, urinary catheters and others. Antibiotic treatment before the isolation with carbapenem, quinolones, aminoglycosides was statistically significant as well. A logistic regression model identified hospitalisation the previous month, incoming due to respiratory disease, No. of comorbidities: 2, respiratory maneuvers and prior carbapenem and aminoglycosides use, among others, as independent risk factors for acquisition of MRPA. Among patients with isolations MRPA were found as factors of poor prognosis the stay in ICUs and onco-haematology services, invasive respiratory maneuvers, smoking among others.
Conclusions: The stay in ICUs and extended hospitalisation are risk factors for acquiring MRPA. It is often associated with clinical respiratory and with a mortality higher than the susceptible strains. The previous contact with the hospital, as well as the respiratory manoeuvres and prior use of certain antibiotics are independent risk factors for the acquisition of PAMR. On the other hand, admission in onco-haematology wards, sepsis and invasive maneuvers as MV ⩾7 days were independent factors of bad pronogsis among patients with MRPA.

P1655 Epidemiological and clinical features associated with the colonisation/infection by *Acinetobacter baumannii* heteroresistant to carbapenems

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Objective: To determine the epidemiological and clinical features associated with the colonisation/infection by *Acinetobacter baumannii* (Ab) Heteroresistant (HR) to Carbapenems (CP).
Methods: Sixty four Ab (identified by ARDRA) representing all the pulsotypes (PFGE-type) and susceptibility patterns to CP (MIC range: ⩽2 to 32 mg/L) observed during November 2000 from 25 Spanish hospitals were included. Isolates were considered HR if there was growth of colonies within the inhibition zone of disks containing imipenem (IP) or MPM (agar diffusion method). Clinical and epidemiological data from patients with Ab HR to CP were compared with those of patients with Ab non HR to CP (prospective cohort study). Chi-square or Fisher test were used in the univariate analysis for qualitative variables. Differences were significative for $p \leqslant 0.05$.
Results: Of the 64 isolates 41 (64%) showed HR to CP and 23 (37%) did not. All the Ab non HR to CP were susceptible to IP (MIC ⩽4 mg/L), whereas 14 (34%) of Ab HR to CP were susceptible to IP. Fifty nine Ab (38 HR to CP and 21 non HR to CP; 35 different PFGE-types) isolated from 59 patients with available clinical and epidemiological data were analysed. Differences in the predisposing factors between patients with Ab HR to CP and patients with Ab non HR to CP were significative for ICU admission (61% vs 33%; $p = 0.04$), COPD (8% vs 29%; $p = 0.05$) and previous use of penicillins (49% vs 14%; $p = 0.01$). For previous use of CP the differences were 26% vs 5% ($p = 0.07$). The frequency of clinical and outcome variables in patients with Ab HR to CP and in patients with Ab non HR to CP were: infection (65% vs 48%; $p = 0.2$), sepsis (76% vs 70%; $p = 0.6$), respiratory tract infection (56% vs 50%; p=1), urinary tract infection (4% vs 30%; $p = 0.06$), skin and soft tissue infection (28% vs 10%; $p = 0.3$), primary bacteraemia (8% vs 0%; $p = 0.2$), treatment with CP (62% vs 33%; $p = 0.2$), crude mortality (patients with infection: 29% vs 40%, $p = 0.6$; patients treated with CP: 25% vs 33%, p=1; all patients: 22% vs 24%, p=1).
Conclusions: Variables significantly associated with Ab HR to CP isolates were: ICU stay and previous administration of penicillins. Central venous catheter and previous receipt of CP almost reached significance. On the contrary, COPD was more frequent among patients with Ab non HR to CP. Ab non HR to CP more frequently caused UTI. No other clinical differences were found. The low number of cases with infection limits the analysis of the clinical significance of HR to CP.

P1656 A carbapenem-resistant *Acinetobacter baumannii* outbreak in ICU. Diagnostic value of rep-PCR for a rapid characterisation of different genotypes, Montevideo, Uruguay

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Introduction: *Acinetobacter baumannii* (Ab) is the 2nd Gram-negative agent associated with nosocomial colonisation-infection. 20–30% has clinical significance. Multiresistance – included Carbapenems – limits their therapeutic options. Control measures require recognition of resistance and genotypes profiles. Molecular methods (PCR and rep-PCR) are useful for typing and detect resistant mechanisms.
Objective: To describe the emergence of Carbapenem multiresistant Ab (CRAb), resistant phenotypes, clinical significance and rep-PCR genotypes
Methods: Prospective observational surveillance study of 390 ICU (22 beds) consecutive patients admitted in January-October 2007.
Ab identified by conventional methods and API NF ®. Susceptibility test: disk diffusion agar CLSI 2007 and Minimal Inhibitory Concentration (MIC) by E-test ®. Carbapenemase activity: Hodge Test, MBL E-test®, and blank disks with EDTA-mercaptopropionic acid. Genotyping by rep-PCR and electrophoresis in polyacrylamide gel.
Results: Overall: Ab isolated in 51 patients from 390 admissions: Blood 1, Spinal fluid 3, Lower respiratory tract 35, Wound 5, Urine 6, Catheter 1. Clinical significance: 13 (25%).
Period 1) Until June all (12) were Carbapenems susceptible:MIC ⩽1 mg/L, and lower MICs (<1.5) to Tygecicline (TYG), Minocycline (MIN) and Colistin; Resistant to Ceftazidime MIC > 256 mg/L, Ciprofloxacin MIC > 32 mg/L and 50% Amikacin resistant. Clinical significance: 3 (25%): 1.8 Ab infections 1000 patients-day. Rep-PCR genotypes: 2 profiles A-B.
Period 2) June-October: 39 Ab. Clinical significance: 10 (25.6%): 5.0 Ab infections 1000 patients-day. From June an outbreak of CRAb (25) emerges. Low level resistance: Imipenem MIC range 2–8 mg/L, Meropenem MIC range 4–32 mg/L, susceptible to TYG, MIN, COL and to Sulbactam in MIC range 8–64 mg/L. Hodge test, MBL E-test® and inhibition EDTA disks: negative for carbapenemase activity. Rep-PCR: new profile C. (First case from Argentine frontier). Remarkable relationship between mortality and clone C: 8 (all) patient dead associated with this genotype.
Conclusion: Outbreak and rapid spread of a CRAb clone associated with mortality. Resistant phenotypes profiles not related to (MBL) metaloenzymes, not being able to exclude OXA type enzymes. Rep-PCR is useful for surveillance; the results were soon available differentiating an emerging clone from endemic.
The low values observed in Carbapenem MICs may be incorrectly identified by agar disk diffusion tests.

P1657 **A new fear for intensive care unit patients: colistin-resistant MDR Gram-negatives**

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Objectives: Selective pressure due to extensive colistin (Col) use seems to lead to the emergence of Col resistance among Gram(−) strains, jeopardising treatment choices in the ICU. The aim of this study was to present the emergence of Col R Gram(−) strains isolated from ICU patients in association with the exposure to Col.
Methods: The study was performed in a 12-bed University General ICU from Nov 2003 to Dec 2006. Empirical antimicrobial treatment was guided by weekly active surveillance of patients' floras. All specimens were cultured in MC agar plates containing antibiotics in order to focus on resistant pathogen detection. Col R was defined by Etest according to the BSAC breakpoints (>4 μg/ml). Epidemiological typing was performed by REP-PCR in the fist sensitive and the first Col R isolate from each patient.
Results: 152 patients were included in the study. Patients were colonised by *K. pneumoniae* (54%), *P. aeruginosa* (57%), A.baumanni (75%), *Enterobacter* spp. (17%), *E. coli* (28%) and *S. maltophilia* (30%) while ColR strains were 37%, 4.5%, 2.5%, 4%, 7.3% and 44% respectively. Among patients colonised with at least one ColR strain, 95% had been exposed to Col while among patients who had been exposed to Col, 66% developed at least one ColR strain. Epidemiological typing demonstrated 20 distinct clones of ColR *K. pneumoniae* isolated from 30 patients. The median duration of exposure to Col, for patients that were colonised or infected by distinct ColR *K. pneumoniae* clone during their hospitalisation was 18 days. For 6 patients whose sensitive and resistant isolate belonged to the same clone, the median duration of exposure was 16 days. 6 patients had exposed to Col ⩽4 days (0–4). In all of these cases epidemiological typing showed that there was horizontal transmission of the resistant clone among co hospitalised patients. Patients that were colonised by *K. pneumoniae* ColR strains were colonised also by ColR A.baumannі and *P. aeruginosa* strains. 18.5% of patients were also colonised with an intrinsically ColR enterobacteriacae; among them 93% had been exposed to Col ($p < 0.01$, OR:11.66). Blood stream infections(10) and VAP(2) from intrinsically ColR enterobacteriacae were reported only in patients exposed to Col.
Conclusion: The treatment choices for infections caused by MDR pathogens in ICUs are already limited. Unnecessary and/or prolonged (>2weeks) COL administration should be eliminated due to increasing resistance to Col among Gram(−) strains.

P1658 **Antibiotic resistance in *Pseudomonas aeruginosa* from food-producing animals: is it a threat for hospital-acquired infections?**

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Objectives: It is well known that injudicious use of antibiotics in humans plays a major role in developing antibiotic resistance in hospital bacteria, however, there is controversy surrounding the use of antimicrobials in food-producing animals, and their adverse effect on humans. In addition, there is clear evidence that in the case of zoonotic bacteria direct transfer to humans has taken place, but these pathogens are not a problem in hospitals. The real threat comes rather from multi-resistant clinical bacteria such as *Pseudomonas aeruginosa*. In this context, the aim of this study was to investigate whether there is a link between antibiotic-resistant *P. aeruginosa* isolated from food-producing animals and human isolates.
Methods: Samples were taken from 31 farms and two abattoirs. Samples (1034) were collected from cattle, sheep and pigs. In addition, 30 meat products were bought from supermarkets and local shops. *P. aeruginosa* clinical isolates (29) were obtained from two major Scottish hospitals. Ten grams of faeces and food were homogenised in peptone water and inoculated into acetamide broth then incubated for 24 hrs at 37ºC. This was spread (500μl) onto cetrimide agar and incubated at 42ºC. Suspected isolates were characterised by the API 20NE test, and polymerase chain amplification (PCR) of 16s rDNA was used to identify *P. aeruginosa*. The minimum inhibitory concentration (MIC) of antibiotics was determined by the agar dilution method following the BSAC Guidelines. Pulsed-field gel electrophoresis (PFGE) typing was performed using SpeI restriction endonuclease.
Results: Out of 1034 animal samples processed, 40 (3.96%) were positive for P. aeriginosa. There were 25 isolates from pigs, of which 7 (28%) had low-level imipenem resistance (LIR) (MIC 8–16 mg/L), 8 from sheep and 7 from cattle. All isolates were sensitive to ciprofloxacin, ceftazidime, piperacillin/tazobactam and meropenem. No carbapenemase genes were detected in LIR. The PFGE showed that out of 40 animal isolates 29 (72.5%) grouped in 8 clusters, with one cluster containing LIR isolates; while out of 29 (6.89%) human isolates only 2 grouped in one cluster with the remaining 27 isolates constituted individual profiles. Not a single human isolate grouped with the animal cluster.
Conclusion: We showed that only few animal isolates had LIR but all were sensitive to other major antibiotics. In addition, all animal isolates were genetically distinct from human isolates by PFGE.

Molecular typing on a per species basis

P1659 **Whole genome typing strategy for phylogenetic assignment of species**

S. Panaiotov, V. Levterova, N. Brankova, K. Tankova, T. Kantardjiev (Sofia, BG)

Amplified fragment-length polymorphism (AFLP) is a whole genome typing technique based on selective amplification of digested DNA in order to create fingerprint for a particular genome. AFLP is utilised for typing of microbial organisms, genealogical studies and phylogenetic studies. AFLP technique generates large numbers of specific marker fragments that could be used for phylogenetic assignment.
Objectives: To develop methodology that assigns phylogenetic relatedness between known genetic patterns and species under study.
Methods: More than 150 mycobacterial isolates, 80 fungi and other 120 bacterial species have been tested. BamHI, PstI, MboI, HindIII, HaeIII and XhoI restriction enzymes, appropriate adaptors for the restriction sites, primer variations and amplification conditions have been tested to identify characteristic AFLP species-specific patterns. AFLP fragments were separated on ALFexpress II sequencer and data elaboration with GelCompar II software.
Resutls: Best results were obtained with PstI and BamHI restrictases, adaptors ligated by T4 ligase to the restriction sites and fragments amplified with PstI and Cy5 labeled BamHI primers. We demonstrate species have unique AFLP pattern. Database of AFLP patterns has been created and in use for identification of new AFLP patterns.
Conclusions: Commercially available AFLP kits use EcoRI and MseI restrictases. The fragments are amplified by using A-, T-, G- C- or combinations for selective fragment amplification. As a result only the number of the amplified fragments is reduced and no additional polymorphic sites are found. More restriction enzymes should be investigated to identify maximum valuable polymorphisms. To obtain good and reproducible AFLP profiles it is essential that DNA is isolated from fresh cultures and free of RNA. Residual RNA molecules could seriously compromise PCR amplification. Residual non ligated adaptors inhibit AFLP analysis. Comparison of different AFLP patterns could identify genus, species, and strains' specific bands. For phylogenetic studies and identification purposes, genus and strain specific bands are of major interest. For epidemiological strains' typing, of major interest are the highly polymorphic strain specific bands. AFLP analysis might prove to be a reliable tool for identification and typing of unidentified isolate(s) and assignment of phylogenetic position. Establishing an AFLP database one can identify isolates and assign phylogenetic relations.

P1660 **Assessment of Bordetella pertussis genotypes distributed in Bulgaria by direct analysis of clinical samples by multiple-locus variable-number tandem repeat**

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Bordetella pertussis, the causative agent of whooping cough is endemic in Bulgaria despite extensive nationwide vaccination since 1950s. It is suspected that strain variation plays a major role in the upsurges of the disease. We applied multiple-locus variable-number tandem repeat analysis (MLVA-VNTR) for typing of B. pertussis directly in clinical samples.

Objectives: To study the genotypes of B. pertussis distributed in Bulgaria applying MLVA typing strategy. The pertussis genotypes identified in Bulgaria were also compared with those found in a selected Italian samples.

Methods: More than 400 clinical samples were tested for the species-specific IS1111 target of B. pertussis by PCR. Swabs were collected from children (age 0–15) suspected for pertussis. Positive by PCR samples were selected for typing according to the quantity of DNA available from the swabs. 35 positive samples were typed for the variability of four VNTR markers, following methods already described. Ten Italian samples were used to compared the results. Each VNTR locus was amplified using fluorescently labelled primer. In order to reduce the number of PCR reactions two VNTR loci were amplified by multiplex PCR. Simultaneous amplification of all four VNTR loci was not manageable directly on clinical swabs. The Shannon-Weiner diversity index was calculated for each locus and ranged from 0.5 to 0.7. In one sample duplication of VNTR3 locus yielded two fragments differing in size of 5 bases. The relationship between various genotypes is visualised by constructing a minimum spanning tree and a dendrogram.

Results: Genotypes in both countries differ, but one prevalent genotype could be identified. 9 VNTR polymorphisms and 4 genotypes were identified. More VNTR loci should be added to increase the discriminatory power of the methodology.

Conclusions: These preliminary results seems to be very promising to understang the biodiversity of pertussis strains circulating in our country. VNTR could be applied as screening strategy in identifying outbreaks and epidemiological studies.

P1661 **Characterisation of an emm12/T12 GAS clone associated with paediatric invasive disease in Portugal**

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Objectives: We have previously reported a significant association between a clone presenting the emm12/T12 gene and paediatric invasive infections among a collection of 160 Group A Streptococci (GAS) isolates recovered from normally sterile sites in Portugal, during 2000–2005. The aim of the present work is to further characterize this clone with respect to antimicrobial susceptibility and to the presence of several genes encoding streptococcal exotoxins and fibronectin- or collagen-binding proteins.

Methods: All isolates (n = 10) were characterised by T typing, emm typing, pulsed-field gel electrophoresis (PFGE) macrorestriction profiling, and multilocus sequence typing. Susceptibility to penicillin, clindamycin, erythromycin, and tetracycline was tested by disk diffusion. The presence of the streptococcal exotoxin genes speA, speB, speC, speF, speH, speI, speK, speJ, ssa, and smeZ, and of the fibronectin- or collagen-binding proteins encoding genes prtF1, prtF2, and cpa was assessed by PCR with specific primers.

Results: During 2000–2005, ten emm12/T12 isolates were identified among a collection of 160 GAS strains isolated from invasive infections in Portugal, of which seven were recovered from paediatric patients ($\geqslant$18 years), showing a statistically significant association between emm12/T12 isolates and paediatric invasive disease ($p = 0.002$, one-tailed Fisher's exact test). All isolates were susceptible to the antimicrobial agents tested and were included in the same PFGE clone (Dice coefficient $\geqslant$80% in an UPGMA dendrogram), which is characterised by T12, emm12, ST36, and by the presence of the gene speH (three isolates were also positive for speC).

Conclusion: An association of a clone defined by a characteristic PFGE profile, emm12, T12, ST36, and carrying the speH gene, with paediatric invasive disease was observed in Portugal. A high frequency of carriage of this clone in children may contribute to this association, as well as a preference of this clone for colonising or causing infections in children rather than adults. The association between emm12 and paediatric invasive infections was previously reported in the Netherlands, although the data available does not allow us to conclude if it was the same clone as the one reported here.

P1662 **Molecular epidemiology of clinical isolates of *Streptococcus pneumoniae* at a children's hospital in Turkey**

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Objectives: Pneumoccal diseases are the leading vaccine preventable cause of childhood deaths. Pneumococcal vaccines are not in routine use as part of the national immunisation programme for children in Turkey. Effective surveillance of pneumococcal isolates can assist in evaluating the necessity of pneumococcal vaccines in Turkish children. This study investigates the molecular epidemiology of *Streptococcus pneumoniae* strains isolated from children in Ankara, Turkey.

Methods: Clinical isolates were obtained from children attending Hacettepe University Ihsan Dogramaci's Children Hospital in Ankara, Turkey. Multilocus sequence typing (MLST) was performed for a total of 96 *S. pneumoniae* strains. All isolates were serotyped by multiplex-PCR and quellung reaction with specific capsular antisera of *S. pneumoniae*.

Results: All isolates were found in 34 different serotypes and 6 isolates were untypeable. The most frequent serotypes were 19F, 14, 6A, 6B, respectively. Thirty-seven (38.5%) isolates were 7-valent (excluding 6A) vaccine types. Only three of the isolates were invasive. MLST analysis of isolates demonstrated a diversity of strains as 69 different allelic profiles were present. Thirty-seven (38.5%) new STs were determined. MLST revealed that 28 (29.2%) of the isolates were related to 7 international clones identified by Pneumococcal Molecular Epidemiology Network. These seven clones were England14–9 (n = 4), Spain23F-1 (n = 7), Spain6B-2 (n = 4), Spain9V-3 (n = 4), Portugal19F-21 (n = 4), Taiwan19F-14 (n = 4), Taiwan23F-15 (n = 1). Evidence of serotype replacement was observed in the isolates related to the England14–9 and Spain23F-1 clone, which indicates capsular switching.

Conclusion: The coverage of the 7-valent vaccine was low with only 38.5% of strains included. Among the 69 MLST STs found in these 96 isolates, 37 new STs were identified and strains belonging to well described international clones were also present. This suggests that the distribution of strains in Turkey differs somewhat from those encountered in other countries and that multiple clonal spread is occurring within the region. This study may provide an important baseline to assess the future use of pneumococcal vaccines in routine vaccination programmes in children in Turkey.

P1663 **Clonal complexes causing invasive meningococcal disease in the Czech Republic in 2005–2007**

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Objectives: Surveillance of invasive meningococcal disease (IMD) has been performed in the Czech Republic since 1993. Molecular methods of characterisation of *Neisseria meningitidis*, which have been used continuously in the National Reference Laboratory for Meningococcal Infections (NRL) allow precise assessment of the epidemiological situation and give a base for precise vaccination strategy in the country. The NRL participates in the EMERT (European Meningococcal Epidemiology in Real Time) project organised by the EMGM (European Monitoring Group on Meningococci) with data submitted from January 2007 (http://emgm.eu/emert/).

Methods: All isolates from IMD sent to the NRL were characterised, with a total of 136 *N. meningitidis* isolates causing IMD in the Czech Republic in the period 2005–2007 (October). The isolates were characterised by serogrouping, serotyping, PorA and FetA sequencing (http://neisseria.org/nm/typing/) and multilocus sequence typing (MLST) (http://pubmlst.org/neisseria/).
Results: Serogroup B (MenB) was prevalent (66.2%), followed by serogroup C (MenC) (29.4%). Among MenB isolates, 52 sequence types (STs) were identified, 29 of them were new, described for the first time on Czech isolates. These 52 STs belonged to 12 clonal complexes, 17.8% of MenB isolates were unassigned to clonal complexes. The most frequent clonal complex among MenB isolates was cc41/44, followed by cc32, cc18 and cc269. Among MenC isolates, 12 STs were identified, 6 of them were new. These 12 STs belonged to 4 clonal complexes. 2.5% of MenC isolates were unassigned to clonal complexes. The most frequent clonal complex among MenC isolates was cc11, followed by cc41/44. PorA and FetA sequencing showed lower heterogeneity of MenC isolates compared to MenB. Five isolates of serogroup Y were found and two of them belonged to the hypervirulent cc23. Only one isolate of serogroup X was detected, not belonging to a clonal complex.
Conclusion: *N. meningitidis* B isolates were prevalent and showed higher genetic heterogeneity compared to *N. meningitidis* C isolates. Among all isolates two clonal complexes prevailed: cc11 (22.1%) and cc41/44 (21.3%).
Acknowledgement: This work was supported by Ministry of Health, by research grant 1A8688–3 and made use of the Multi Locus Sequence Typing website (http://pubmlst.org/neisseria/) sited at the University of Oxford and funded by the Wellcome Trust and European Union.

P1664 Design and implementation of an external quality assurance scheme for *Neisseria meningitidis* characterisation for cultures and non-culture material

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An external quality assurance (EQA) scheme was designed to optimise and standardise European meningococcal reference laboratory meningococcal strain characterisation and detection to ensure accurate surveillance.
Three panels of *N. meningitidis* isolates and simulated septicaemic blood samples were distributed for phenotypic and genotypic (meningococcal nucleic acid detection, serogroup determination and sequence typing) characterisation as performed by EU-IBIS participating Reference laboratories. Isolates were selected to represent important circulating strains in Europe. The simulated septicaemic blood samples comprised "standardised" heat-killed suspensions of organisms diluted in defibrinated horse blood prepared by the HPA Meningococcal Reference Unit. Freeze-drying, panel distribution and reporting were carried out by the HPA Quality Assurance Laboratory.
Each participating laboratory received a report comparing their results to the consensus for each sample characteristic. There was good agreement for the phenotypic characteristics. Minor clerical errors in reporting and some differences in nomenclature were identified. In those laboratories undertaking genotypic characterisation (including porA sequencing and Multilocus Sequence Typing) there was excellent agreement but notable differences with the weaker positive (lower copy number) material were observed.
European phenotypic and genotypic strain characterisation including nucleic acid detection and non-culture genotyping was successfully established for EU-IBIS participants. The EQA distributions demonstrated the utility and improved standardisation obtained by molecular strain characterisation. The second EQA distribution identified areas for improvement primarily concerning low copy number nucleic acid detection for non-culture confirmation of infection and subsequent strain characterisation including serogroup identification. Also highlighted were issues regarding reporting, nomenclature and reagent availability. The second distribution informed cthe design of the third distribution, the results of which are awaited.

P1665 Population structure of *Enterococcus* isolates from Portugal

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Objectives: Portugal is one of the EU countries with highest vancomycin-resistant enterococci (VRE) rate. Our aim is to analyse the population structure of enterococci and their relationship with the spread of glycopeptide resistance.
Methods: We studied 24 *E. faecalis* (Efc) and 24 *E. faecium* (Efm) isolates of a large Portuguese collection (1996–2004) from nosocomial infections (H, n = 32; from 3 cities), environment (E, n = 3), healthy humans (C, n = 5) and animals (swine-S, n = 1; poultry-P, n = 7). Clonal relatedness was established by PFGE and MLST. Virulence traits (gel, asa, cyl, hyl and esp) were searched by PCR. Analysis of GRE included characterisation of Tn1546 backbone (PCR overlapping; sequencing), transferability and genomic location (I-CeuI/S1 nuclease; vanA/23S rDNA probes).
Results: Efc strains from hospitalised persons were classified as high-risk enterococcal clonal complexes (HiRCC) CC2 (ST6) or CC21 (ST21, ST22, ST117), and singletons ST159 (a new ST located in two cities 4 years apart), ST19, ST30, ST41, ST55, most observed in both VRE and VSE strains. STs from other sources were ST16 (C, P), ST35 (P), ST26 (C) and the new ST160 (C). Most Efc contained gel, asa, cyl and esp (95%, 89%, 53% and 47%, respectively). Six Tn1546-types were detected, although variant PP4 containing ISEf1 confined to H and located on large transferable plasmids (84–145 kb) was the predominant. Most VREfm strains (17/18) from H, E and C belonged to the widely disseminated CC17 (ST18, ST80, ST280, ST16, ST132, ST366, ST367, ST368 and ST369). ST366-ST369 were identified in H and E and assigned as new variants of CC17. CC5 (ST6) was observed in 2 VRE strains from H and S, both containing Tn1546-type D. All VREfm isolates from P were grouped in ST9 (n = 2) and ST12 (n = 3). Efm strains from H and E carried only esp gene (50%) or hyl (19%). PP-4/PP-5 ISEf1-carrying Tn1546-types from H were also predominant among Efm strains and located on large conjugative plasmids (80–110kb).
Conclusions: Our results confirm the relevance of the worldwide spread Efc and Efm HiRCC. Dissemination and persistence of ST159-Efc among Portuguese hospitals might reflect the emergence of a new epidemic CC. The similarity of Tn1546 and plasmid profiles found among different STs indicates that horizontal transfer also plays a relevant role in VRE spread in Portugal.

P1666 Clonal analysis of vancomycin-resistant *Enterococcus faecium* by MLVA and PFGE typing

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Objective: The aim of the study was to reveal the clonal structure of a vancomycin-resistant *E. faecium* (VREM) population by MLVA and PFGE approaches and to compare these methods in an epidemiologic investigation.
Methods: This study was performed on 121 VREM isolates collected from several Polish hospitals in the National Medicines Institute in Warsaw between January 2006 and November 2007. The VREM were obtained from rectal swabs (n = 91), wound lesions (n = 7), blood (n = 13), urine (n = 8) and CSF (n = 2). PCR for van genes was performed for all isolates, followed by MLVA and PFGE (SmaI digest) typing.
Results: There were 35 vanA and 85 vanB genotypes detected among the isolates. In 2007 90% of isolates were vanA-positive, in contrast with 2006, when only 9.3% of isolates had the vanA genes.MLVA and PFGE resulted in 35 and 21 types of 119 and 117 typeable isolates, respectively. Comparable values of the discriminatory power, 0.6391 for MLVA and 0.6309 for PFGE, were observed. Among the determined MLVA types (MTs) 12 were new. MT159 was predominant in 2007, representing 77.9% isolates from that year. The most of 2007 isolates (74.7%) belonged to 15 subtypes of the PFGE type PT1, which comprised 92.5% of the MT159 isolates. Both MLVA and PFGE revealed greater genetic diversity among 2006 isolates (12 MTs and 22 PTs among 30

isolates) than among those from 2007 (11 MTs and 15 PTs among 91 isolates). No predominant MLVA or PFGE type was detected in 2006. The majority of isolates from 2006 and 2007 (>70% and >90% respectively) were classified into the CC17 clonal complex based on allele profiles determined by MLVA.

Conclusion: MLVA appeared to be comparably usefull to PFGE in the epidemiologic analysis of VREM. Both methods reflected highly concordant data concerning the level of genetic diversity among the investigated isolates.

P1667 Evaluation of multi-locus VNTR sequence typing for clustering of vancomycin-resistant *Enterococcus faecium* clinical isolates

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Objectives: Vancomycin-resistant *Enterococcus faecium* (VREF) cause nosocomial infections especially in compromised patients. Their molecular epidemiology is primarily characterised by pulsed-field gel electrophoresis (PFGE). However, this method is very laborious and difficult to standardize for intra- and inter-laboratory comparisons. Recently, MLST (multilocus sequence typing)- and MLVA (multilocus variable-number of tandem repeats)-schemes were developed to overcome these drawbacks. Due to their low discriminatory power their applicability in outbreak investigations is limited. Therefore, we evaluated a multilocus VNTR (variable number of tandem repeats) sequence typing (MLVST) scheme for VREF.

Methods: To identify suitable repeat regions for MLVST, we analysed the genome sequence of *E. faecium* DO with the programme 'Tandem Repeats Finder'. From a total number of 1580 repeat regions, three were finally selected for further analysis (VNTR0032, VNTR0047, and VNTR1398). The corresponding regions were sequenced from a well-characterized reference collection comprising 144 environmental and clinical VREF strains. To evaluate MLVST, the discriminatory index (DI), the concordance to MLST and MLVA was determined for different VNTR combinations, and tested in routine diagnostics using 47 clinical isolates from related and unrelated strains based on PFGE data isolated at the University Hospital Münster, Germany.

Results: Preliminary tests with the strain collection demonstrated that the three VNTRs displayed individual DIs of 0.718 (VNTR0032), 0.794 (VNTR0047), and 0.835 (VNTR1398), whereas MLST and MLVA resulted in DIs of 0.971 and 0.935, respectively. Typeability was 100% (VNTR0032), 91.67% (VNTR0047), 84.03% (VNTR1398), 100% (MLST), and 80.56% (MLVA). Analysis between the combination of all three VNTRs and MLST/MLVA resulted in concordances of 93.1%/91.6%. The combination VNTR1398/atpA/ddl (both genes from the MLST scheme) accomplished the highest DI value of 0.974 and was used for further analysis. Applying MLVST on the 47 clinical isolates, 43 were clustered concordant to PFGE grouping.

Conclusion: VNTR regions are appropriate targets for sequence-based typing approaches. MLVST is a faster and less cost-intensive alternative typing method for VREF in comparison to MLST and results in clustering concordant to PFGE. Ongoing studies investigate the performance in comparison to PFGE and the applicability in daily clinical routine on a broader scale.

P1668 Genetic characterisation of vancomycin resistant isolates of *Enterococcus faecium* isolated from patients in Tehran

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Objectives: We have previously found that up to 12% of clinical isolates of *E. faecium* at Tehran hospitals showed resistance to vancomycin. However, little data was available on the genetic heterogeneity of vancomycin resistant isolates (VRE) among the Iranian population of this organism. Therefore the emergence of infections with the new clones of VRE needs to be investigated.

Materials and Methods: Collectively, 103 non replicative isolates of enterococci grown from urine samples in the first quarter of 2005 were screened for their susceptibilities to different antibiotics. Ribotyping was then used to genetically characterize the isolates of VRE.

Results: Using disk diffusion method, all isolates were found susceptible to linezolid and nitrofurantoin. All isolates of *E. faecalis* (n = 86) were susceptible to vancomycin. Conversely, over 70% of *E. faecium* isolates (n = 12) showed resistance to this glycopepide. The VRE isolates recovered from patients in 2005 were heterogenous comparing with those of 2000.

Conclusion: Conventional bacteriology confirmed the increase in rate of VRE. It appears that a variety of new VRE clones have arisen recently at different wards of this hospital as determined by ribotyping.

P1669 Identification of enterococcal plasmids by multiplex-PCR-based relaxase typing

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Objectives: Identification of plasmids is important in order to analyse their distribution and relationships to host cells, their evolutionary origins, and the horizontal gene transfer, a process with a great impact in the spread of antimicrobial resistance. Although methods for the identification of plasmids from enterobacteria were soon developed, no such methods are currently available for enterococcal plasmids. We have developed a novel PCR-based typing method to describe the ecology of the antibiotic resistance plasmids in *Enterococcus* and evaluate their role in the spread of antimicrobial resistance. Since plasmid transfer is an almost universal procedure for gene spread among bacteria, a typing method based in the relaxase, the crucial protein involved in initiation of transfer, could be widely applicable.

Methods: 14 pairs of primers were designed on the basis of published enterococcal relaxases sequences. 27 reference enterococcal plasmids/strains were first tested using simplex-PCR with each pair of primers for initial testing of the oligonucleotides. 3 Multiplex-PCRs were then performed on the reference plasmids/strains. Sequencing of the amplicons was done by standard procedures for confirmation of the results.

Results: The specificity of each primer pair was tested on two plasmid-free *Enterococcus* strains: *E. faecium* BM4105RF and *E. faecalis* UV202. None of the primer pairs gave any amplification using both templates. Multiplex 1, 2 and 3 correctly detected the relaxases encoded on the reference plasmids and no false positive results were observed.

Conclusions: The method developed is specific and sensitive and does not require a laborious work. Individual or multiple plasmids present in the same cell can be easily detected. Therefore, this approach is potentially applicable for the typing of known enterococcal plasmids present in diverse collections of strains. It will facilitate the understanding of the tremendous spread of the antibiotic resistance in *Enterococcus*.

P1670 Are the days of PFGE numbered? A comparison of PCR-based typing methods with PFGE for identification of nosocomial transmission of MRSA

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Objectives: Several new PCR-based typing techniques for meticillin-resistant *Staphylococcus aureus* (MRSA) have been recently described as alternatives to Pulse field gel electrophoresis (PFGE) of SmaI digested genomic DNA. However, their relative utility for identifying nosocomial transmission episodes remains controversial. This study sought to determine the usefulness of some of these techniques for this purpose.

Methods: Fifty multiresistant MRSA isolates from epidemiologically confirmed nosocomial transmission events were studied. The isolates came from two Sydney tertiary referral hospitals, where the prevalence of MRSA amongst *S. aureus* isolates is approximately 50%. Additionally, 18 sporadic isolates with the same antibiograms from patients who attended emergency departments or outpatient clinics but who were not

admitted before or since were also collected. PFGE, Multilocus variable number of tandem repeats analysis (MLVA) and spa sequence typing were performed using published methods. Toxin gene profiling (TGP) and SCCmec subtyping were performed using multiplex PCR and reverse line blot assays developed in our laboratory.
The relative performance of the five methods was assessed using Simpson's index of diversity, the mean similarity of each pair of transmission isolates, and the Pearson correlation of the similarity matrix obtained using cluster analysis of the clinical relatedness with the similarity matrix obtained using each typing method (clinical congruence).
Results: The results are shown in the table below. MLVA and PFGE performed equally well in predicting the clinical relatedness of isolates, but were clearly superior to spa typing, TGP and SCCmec subtyping for this purpose. The effect of combining PCR-based methods was also assessed. Combining spa typing, TGP or SCCmec subtyping with MLVA did not appear to improve performance.

Table. Performance of five typing methods in identification of nosocomial MRSA transmission. Values are percentages

	PFGE	MLVA	*spa*	TGP	SCC*mec*
Simpson's Index of Diversity	99	99	83	73	60
Mean similarity	80	70	66	93	93
Clinical congruence	31	31	16	11	14

Conclusions: Of the PCR-based methods studied, MLVA appears to be the best candidate to replace PFGE for the identification of nosocomial transmission episodes of MRSA. However, TGP and SCCmec subtyping may be useful to provide molecular markers of clinically and epidemiologically relevant phenotypic characteristics.

P1671 Detection and genotyping of multidrug-resistant *Acinetobacter baumannii* strains contain multiple blaOXA-like genes in two burn hospitals, Tehran, Iran

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Objectives: This study was designed to detection of multidrug-resistant (MDR) *Acinetobacter baumannii*, distribution of blaOXA-like genes and genotyping analysis of them among burns unit of two hospital of Tehran, Iran.
Methods: during this study the susceptibility of 60 isolates of *A. baumannii* from burn wounds (n = 48), blood samples (n = 4) and trachea (n = 8) was determined with disk agar diffusion method. Also susceptibility to imipenem and meropenem was determined by minimum inhibitory concentration (MIC) according to CLSI guidelines. Distribution of blaOXA-like genes was detected by Multiplex-PCR and then these isolates were typed by PFGE.
Results: Result show that 66.6% (n= 40) of all *Acinetobacter baumannii* isolates were MDR. Colistin and tigecycline were effective against 88.3% (n = 53) and 83.3. % (n = 50) of isolates respectively. 66.6% (n = 40) and 65% (n = 39) of isolates were resistant to imipenem and meropenem in both DAD and micobroth dilution method, respectively (with MIC ranged of 64 to 256). All MDR strains possessed at least two blaOXA-like genes especially blaOXA-51 and blaOXA-23-like carbapenemases. Analysis of PFGE showed the existence of 2 clones (A and B) with different sub clone in both hospitals. Clone A was dominant one of the study hospital was studied.
Conclusion: Our study was shown spread of MDR *Acinetobacter baumannii* with blaOXA-like carbapenemase and similar clonality in burn hospitals of Tehran, Iran. Detection of some strain with high resistance to all antimicrobial agents including carbapenems, colistin and tigecyclin and spread of similar clone in our hospitals suggest that the current protocol for prevention and controlling of infections with these MDR isolates needs to be investigated.

P1672 Molecular epidemiology of *Acinetobacter baumannii* in hospitals in the Mediterranean area using pulsed-field gel electrophoresis and sequence-based typing

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Objectives: To investigate the geographical distribution and genetic relatedness of outbreak isolates of *Acinetobacter baumannii* recovered from the intensive care units of 18 hospitals in Greece, Italy, Lebanon, and Turkey from 1999 to 2006.
Methods: Genotyping was performed by pulsed-field gel electrophoresis (PFGE) analysis of ApaI-digested genomic DNA and sequence-based typing (ST) using outer membrane protein A (ompA), pilus assembly system protein (csuE) and blaOXA-51-like gene sequences (Turton et al., Clin Microbiol Infect, 13, 807, 2007).
Results: PFGE analysis of a collection of 30 *A. baumannii* strains associated with epidemic episodes occurred in four different European countries of the Mediterranean area led to the identification of 12 distinct PFGE profiles. ST analysis assigned 4 of them to ST group 1, 5 to ST group 2, and 2 to ST group 4. ST group 1 and 2 corresponded to the previously identified European clone II and I, respectively, ST group 4 has not been identified before. PFGE profile A of ST group 1 predominated, being isolated in 4 distinct hospitals in Naples, Italy, and in different hospitals of Thessaloniki, Larissa, and Serres, Greece from 2002 to 2006. Additional PFGE types of ST group 1 were isolated in Greece and Italy; PFGE types of ST group 2 were isolated in Greece, Italy, and Lebanon. The two distinct PFGE profiles belonging to ST group 4 were isolated in Thessaloniki, Greece, during 2003, and in Kocaeli, Turkey, during 2005 and 2006. Ten out of 12 PFGE profiles of *A. baumannii* epidemic isolates showed resistance to carbapenems. The blaOXA-58 gene was identified in 9 distinct PFGE profiles of ST groups 1, 2, and 4, and in 4 additional PFGE profiles of either sporadic or micro-epidemic *A. baumannii* isolates from Italy and Turkey; the blaOXA-23 gene was identified only in one epidemic-associated PFGE type of ST group 4 from Kayseri, Turkey.
Conclusions: *A. baumannii* epidemics occurring in Mediterranean hospitals have been sustained by the spread of distinct genotypes belonging to ST group 1, 2 and 4, one single PFGE profile of ST group 1 being particularly successful in Greece and Italy since 2002. The blaOXA-58 gene was present in the majority of carbapenem resistant genotypes.

P1673 Molecular characterisation of carbapenem-resistant *Acinetobacter baumannii* isolates from intensive care unit in a hospital, Bogota, Columbia

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Objectives: Typing molecular from isolates carbapenem-resistant *Acinetobacter baumannii* and to detected microbiological and molecular ocurrence from carbapenemases.
Methods: One hundred thirty two isolates carbapenem-resistant *A. baumannii* from ICU of twelve hospital in Bogotá were collected from 2005 to 2006. The isolates were re-identificated with API 20NE (bioMérieux) and the confirmation of minimum inhibitory concentrations (MICs) of imipenem and meropenem were determined with Etest (AB Biodisk). For detection of metallo-carbapenemases we used MBL Etest strip and gene identification from metallo and oxa-type carbapenemases was by PCR. Amplification products were evaluated by sequentiation.
The characterisation molecular by assessment the relatedness of the isolates was realised by pulsed-field gel electrophoresis (PFGE) of ApaI-digest genomic DNA. The cluster analysis was realised with Fingerprinting II software (Bio-Rad).
Results: All Isolates were resistant to meropenem with MIC > 32 mg/mL and 131 resistant to imipinem with MIC > 32 mg/mL and one isolate showed had intermediate resistant with MIC 8 mg/mL. From isolates

studied only thirty isolates were positive for detection MBL for E-test but neither had VIM or IMP genes by PCR. PCR detection from OXA-carbapenemases gene suggested presence from gene blaOXA-51-like in all isolates. In another hand we founded blaOXA-23-like in 129 isolates, blaOXA-24-like only in one isolate and in the case blaOXA-58-like none amplificated. Sequencing from amplification products confirmed the presence from OXA-72 carbapenemase in one isolate and OXA-23 carbapenemase in the others isolates.
Typing results showed presence a predominant clone conformed by 88 isolates from all hospitals, the others isolates were distributed the following form 3 clones had 2, 5 and 6 isolates each one and two clones conformed by 3 isolates and finally, 14 isolates unique fingerprints.
Conclusions: our results indicate the ocurrence from a predominant clone from *A. baumannii* Carbapenems resistant, this clone were disseminated in all ICU Hospitals from Bogotá studied.
Carbapenemase OXA-23 was the most frecuently founded in our isolates, while OXA 72 was detected in only one intermediate resistant isolate to Imipenem, this is the fist report from this class from OXA-types Carbapenemases from Colombia.

P1674 A multi-locus sequence typing scheme for *Stenotrophomonas maltophilia*

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Stenotrophomonas maltophilia is ubiquitous in nature, but also a highly resistant pathogen and cause of nosocomial infections. In the past, there have been several changes in the taxonomy of *Stenotrophomonas* spp. and delineation of *S. africana* from *S. maltophilia* has been debated. Using Amplified Fragment Length Polymorphism (AFLP), strains were assigned to genogroups.
Apparently, isolates of environmental and clinical origin predominate in different groups.
The MLST scheme was developed on the basis of a selected highly diverse *S. maltophilia* strain collection comprising 52 strains of clinical and 16 strains of environmental origin from seven countries in all continents. They included the type strains of *S. maltophilia*, *S. africana*, *S. acidaminaphila*, *S. nitrireducens*, as well as strains belonging to the 10 different *S. maltophilia* genogroups, as previously defined on the basis of AFLP-patterns.
Compared with all *S. maltophilia* isolates, the DNA similarity of *S. africana* was on average of all seven loci 95.8%, which was above the average of all *S. maltophilia* strains (95.1%). Following this it was considered that the *S. africana* type strain belongs to the species *S. maltophilia*. Sequence data on 70 *S. maltophilia* strains, including the strains R551–3 and K279a from genome sequencing projects, were assigned to 54 sequence types (ST) based on the allelic profiles in the seven loci (atpD, gapA, guaA, mutM, nuoD, ppsA and recA). Analysis of strains from five outbreaks demonstrated the stability of the allelic profile over at least a 90-day period. A neighbour-net presentation of the concatenated sequences confirmed previously defined nine genogroups and revealed five additional groups. 18 out of 31 isolates from respiratory tract specimens, including 12 out of 14 isolates from cystic fibrosis patients, belonged to genogroup 6. However, 16 invasive strains isolated from blood cultures belonged to nine different genogroups. Three genogroups contained isolates of strictly environmental origin only.
This MLST scheme for *S. maltophilia* presents a discriminatory typing method with stable markers appropriate for studying the population structure. According to DNA similarities *S. africana* belongs to the species *S. maltophilia*. MLST data confirmed the existence of previously defined genogroups and an additional five genogroups on the basis of the isolates examined in this study. The predominance of clinical isolates in genogroup 6 requires further elucidation.

P1675 Pulmonary multiple colonisation of distinct genotypes of *Pseudomonas aeruginosa* in a patient admitted to an intensive care cardiology unit: use of a combined fingerprinting approach by rep-PCR and f-AFLP

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Objectives: The human opportunistic pathogen, *Pseudomonas aeruginosa*, is a major cause of infectious-related mortality and morbidity among critically ill patients, and carriers the highest case fatality rate of all Gram-negative infections. In our study, we monitored a multiple *P. aeruginosa* colonisation, occurred in a patient affected by chronic illness, that led us to isolate 12 strains showing different susceptibility patterns and morphological traits, probably associated to distinct genotypes. In order to study their genetic relationships we characterised the entire *P. aeruginosa* panel of strains at molecular level.
Methods: Susceptibility patterns were performed by using VITEK 2 Automatic System (BioMerieux). *P. aeruginosa* genotypes were analysed by, the fluorescent-AFLP (f-AFLP) method (AFLP Microbial Fingerprinting; Applied BioSystem) as reference system and the rep-PCR based on DiversiLab platform (Biomerieux) as rapid but non-standardised typing method. Bioinformatics analysis of f-AFLP traces were performed by BioNumerics Software v. 5.00 (Applied Maths) while the web-based DiversiLab Software (v. 3.3) was used for the analysis of rep-PCR fingerprinting.
Results: Drug susceptibility patterns showed three completely different and four highly correlated antibiotypes. The interpretation of f-AFLP fingerprintings, highlighted the presence of three distinct clonal profiling, antibiotype correlated. Rep-PCR electropherograms, analysed using the Pearson correlation and the Kullback-Leibler algorithm, provided a high similarity rate amongst all the strains; the threshold, fixed between 96–97%, showed three differentially identified groups, consistently with the f-AFLP inferred patterns.
Conclusion: The identification of different clones is a crucial crossing border to establish a correlation between genotype plasticity and resistance patterns in *P. aeruginosa*, especially in patients with life-threatening conditions.

P1676 Molecular epidemiology of *Pseudomonas aeruginosa* from Italian cystic fibrosis patients

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Background: The means of transmission of *Pseudomonas aeruginosa* (PA) in Cystic Fibrosis (CF) affected patients have not been clearly established. Many CF patients appear to acquire the organisms from the natural environment and not from other CF patients (Speert et al., 2002). Other indications, on the contrary, suggest that some clonal lineages are widespread among the CF population, apparently contracted through cross infection from other CF patients.
Aim: Address the type of transmission and/or environmental acquisition of PA and define if epidemic strains are circulating in Italy.
Methods: A collection of 340 strains from 183 pts attending 4 CF centres located in north (A), centre (B) and south Italy (C and D), and 55 environmental (11 from hospital sinks, 44 from swimming pool and mineral water) were genotyped by BOX-PCR (Rademarker J.L.W et al. 1998). A PA panel control strains included: PA01, ATCC 27853, European clone C (EC), Manchester Epidemic Strain (MES) and Liverpool Epidemic Strain (LES). The cluster analysis was performed by "Gel Compar II". MLST analysis of major clonal group was performed as described by Curran et al. 2004 (http://pubmlst.org/paeruginosa).
Results: A total of 179 genotypes were found. 132 pts were colonised with a single PA genotype, and 46 with two o more genotypes. A total of 82 pts showed genotyped "shared" with other pts while 86 pts had "unique" genotype. 36 groups of pts carrying the same genotype (clusters) were observed: 22 of 2 pts size, 7 of 3 pts, 5 of 4 pts and 2 of

5 pts. For environmental strains 25 genotypes were found: 12 not shared, while 13 shared.13 clusters were found: 10 of 2 and 3of 3 sources. No Italian PA CF Strains showed genetically correlation with EC, LES and MES. No genetically correlation was observed between environmental and CF strains. No genotypes correlated were found among strains from Centres A, B and C. 3 genotypes found in 3 pts of centre B are highly correlated with 3 genotypes found in 2 pts of centre D. The profile (acs 39; aro 35; gua 71; mut 81; nuo 56; pps 36; trp 54), obtained by MLST, of this clonal group not showed relationship with European clones.

Conclusions: Environmental acquisition was not documented. The number of cluster found, suggest that person-to-person transmission may occurred, and the need of continuous survey, in the help of the infection control measures.

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P1677 A newly identified clonal *Pseudomonas aeruginosa* strain has a high prevalence in the Dutch cystic fibrosis population

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Objectives: In the last decade, different *Pseudomonas aeruginosa* (PA) clones have been associated with increased colonisation and infection of patients with cystic fibrosis (CF), such as the Liverpool, Manchester and Midlands epidemic strains and Melbourne pulsotype I strain, amongst others. Transmission of these, often virulent, clones between CF patients has been documented as well, which has lead to strict segregation policies for CF patients in many countries, including the Netherlands. However, little information was available on the prevalence and population structure of PA among Dutch CF patients. The aim of this study was to determine the prevalence and genetic linkage of PA genotypes in the CF population of the UMC Utrecht, which contains about 30% of the total Dutch CF population.

Methods: A cross-sectional study was conducted of all CF patients that visited the UMC Utrecht in 2007. The isolates of the first respiratory tract culture yielding PA of each patient were typed with multilocus sequence typing (MLST), as described by Curran et al., including all different PA phenotypes found in this first positive culture. Sequences were analysed in BioNumerics 5.0®.

Results: Of the 1300 CF patients in the Netherlands, 372 (29%) visited the UMC Utrecht in 2007. The prevalence of PA colonisation in this population was 52% (192/372).

140 samples of 95 patients were MLST-typed (results of 100 isolates are pending). We found 63 different sequence types (STs); 50 patients (53%) harboured a unique ST and 45 (47%) carried a strain shared by other patients. 11 STs were documented in 2–4 patients and there was one dominant ST (ST406) documented in 21 of 95 (22%) patients. Based on MLST, this clone is genetically distinct from the known (epidemic) strains.

41 (43%) patients had phenotypically different strains in the sputum culture and in only 5 (12%) patients these strains were also genotypically different.

Conclusion: Approximately 22% of the Dutch CF population harbours the ST406 strain of PA, which differs genetically from the previously reported epidemic clones in other countries. Further research is needed to elucidate why ST406 is highly prevalent in the Dutch CF population.

P1678 Use of MIRU-VNTR typing to differentiate *Mycobacterium tuberculosis* isolates from Bulgaria and comparison with IS6110-RFLP typing and spoligotyping

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Objectives: To assess the discriminatory power of different molecular typing methods (IS6110-RFLP, Spoligotyping, MIRU-VNTR) used alone and in different combinations to evaluate the most appropriate combination and usefulness of VNTRs for molecular-epidemiological studies of *M. tuberculosis* in Bulgaria.

Methods: IS6110-RFLP, Spoligotyping, MIRU-VNTR were used for strain differentiation.

Results: 73 *M. tuberculosis* strains were subdivided into 68 types (21-loci and 15-loci MIRU-VNTR scheme), 59 types (12-loci MIRU-VNTR scheme), 51 types (IS6110-RFLP) and 31 types (spoligotyping). Among 21 tested VNTR loci, we selected 5 loci that allowed to achieve sufficiently high discrimination (52 types). The Hunter-Gaston discriminatory index was as follows: Spoligotyping (0.939), IS6110-RFLP typing (0.983), 12-loci MIRU-VNTR typing (0.992), 5-loci MIRU-VNTR typing (0.988), 21-loci and 15-loci MIRU-VNTR typing (0.997).

Conclusion: We conclude that both MIRU-VNTR typing and spoligotyping methods are rapid, highly reliable and discriminatory methods for epidemiological typing of *M. tuberculosis*; in the Bulgarian setting, MIRU-VNTR typing is the epidemiological method of choice for the near future.

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P1679 Mycobacterial interspersed repetitive units typing reveals high genetic diversity of *Mycobacterium tuberculosis* isolates in Austria

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Objectives: The aim of the study was to determine the suitability of Mycobacterial interspersed repetitive units (MIRU) typing for recognising biodiversity of *Mycobacterium tuberculosis* isolates in Austria. Austria has a total population of 8 million and a yearly incidence of tuberculosis of 11/100,000.

Methods: Mycobacterial interspersed repetitive units (MIRU) typing is a PCR-based typing method which assigns the number of tandem repeats in 12, 15, or 24 independent loci (MIRUs). MIRU-analysis was performed with the 24 MIRU primer system as described [1] on 1294 isolates collected from 2003 to 2005 in Austria. Amplified products were analysed on an automated sequencer. User defined filtering and grouping was performed with an own software tool.

Results: Two of the 24 MIRU-VNTR primers did not generate analysable results. The remaining 22 primer allowed the classification of the 1294 isolates into 1085 different MIRU types. The eight most frequently found MIRU profiles contained 14, 13, 11, eight, and six isolates. All other types were comprised of five or less isolates. The MIRU type comprising 14 isolates was assigned to the Haarlem lineage, the MIRU type comprising 11 isolates was assigned to the Beijing lineage, and one MIRU type comprising six isolates was assigned to the URAL lineage. All of the other frequently found MIRU profiles could not be assigned to known lineages [1].

Eighty-eight of the 1294 isolates were of the Beijing spoligotype; they yielded 71 different MIRU types. One MIRU type was found in 11 Beijing isolates, seven types were found in two isolates each, and 63 types were shown by a single isolate only.

Clonal variants within the same host were detected in 27 out of 44 cases.

Conclusion: Our results highlight the diversity of *M. tuberculosis* isolates in Austria. MIRU is more discriminative than spoligotyping or IS6110 RFLP [2]. MIRU even allows the differentiation of the Beijing family in 71 separate MIRU types. We consider MIRU typing to be a valuable typing method suitable to allow for differentiation between reactivation and reinfection in individual cases, and to provide the discriminatory power necessary to recognize clusters of tuberculosis in Austria.

Reference(s)

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[2] Oeleman MC, Diel R, Vatin V, et al. (2007) J. Clin Microbiol. 45: 691–697.

P1680 The use of MIRU-VNTR for the detection of mixed *Mycobacterium tuberculosis* infection

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Objective: Patients infected with latent *Mycobacterium tuberculosis* (Mtb) can develop disease via either reactivation of the latent bacilli, or by exogenous reinfection with a second mycobacterial strain. The presence of strains with different drug-susceptibility patterns could lead to improper drug-administration and eventually result in treatment failure. The use of molecular typing techniques, such as spoligotyping [2] and IS6110 RFLP [3] generally assume the isolation of only 1 strain from each patient and the detection of a second infection requires a second sampling from the same patient at a later time. Isolates are, based on spoligotyping, grouped into families defined by deletions of particular spacers. Two *M. tuberculosis* families, which are highly prevalent are the T (ST53) and Beijing families [1]. Isolates belonging to the Beijing family shares a highly characteristic pattern with the absence of spacers 1–34 and the presence of spacers 35–43, whereas the T family is poorly defined as only lacking spacers 33–36. Thus, the objective of this study was to investigate for the presence of mixed Mtb subpopulations among cultures from two common mycobacterial families; 1 well-defined and 1 poorly defined, using the 15 loci MIRU-VNTR method.
Methods: Fifty-five isolates (29 ST53 and 26 Beijing) were selected for further characterisation using the 15 loci MIRU-VNTR as described [4] and analysed using the Peak Scanner Software v1.0 (PE Applied Biosystems).
Results: MIRU-VNTR of the 26 Beijing isolates showed only one product for all 15 loci tested, indicating a single Mtb subpopulation. Of the 29 isolates belonging to the ST53 lineage, two PCR products was observed in 7 loci for 2 isolates, 5 loci for 3 isolates, 4 loci for 2 isolates, 3 loci for 2 isolates and 2 loci for 1 isolate. Five isolates produced two PCR products for 1 loci (ETR-A, ETR-C, Qub-11b and 2 for MIRU10).
Conclusion: The 15 loci MIRU-VNTR is highly discriminatory and can, thus, be used to detect mixed Mtb subpopulations. Further characterisation of the isolates that has been classified as belonging to the ST53 lineage by MIRU-VNTR may be useful to give a more precise estimate of the prevalence of this family.

Reference(s)

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P1681 Establishment of molecular typing scheme for determination of recurrence and reinfection of *Mycobacterium tuberculosis*

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Objectives: The purpose of this study was to establish a typing scheme to distinguish between recurrence and reinfection of *Mycobacterium tuberculosis* (MTB).
Methods: Four hundreds and nine MTB isolates from 323 patients were typed by two different molecular typing methods including mycobacterial interspersed repetitive unit (MIRU) and spoligotyping. Nineteen loci were selected for MIRU typing, and a commercial kit was used for spoligotyping. Patients with multiple MTB isolates of different types were considered reinfected, and those with multiple isolates of the same types were considered as having recurrent infection or incomplete-treatment.
Results: By spoligotyping, 229 of the 409 (56.0%) isolates were determined to belong to the Beijing genotypes, and the other 180 (44.0%) were non-Beijing genotypes. A total of 160 MIRU types were identified among the 409 MTB isolates. 66 patients were found to have more than one MTB infection and therefore had more than one MTB isolate. 51 of these patients had multiple MTB isolates with the same genotypes and antibiotic susceptibility patterns. The other 15 patients had multiple MTB isolates with different MIRU types or antibiotic susceptibility patterns, including 4 with isolates with different MIRU genotypes, 8 with isolates of different antibiotic susceptibility patterns, and 3 with isolates that were different in both genotypes and antibiotic susceptibility patterns. Therefore, 7 of these patients (7/66; 10.6%) were classified as reinfected and the other 59 (89.4%) belonged to the recurrence or incomplete-treatment group. Isolates with antibiotic susceptibility changed from susceptible to resistant were mainly from patients treated with isoniazid and rifampin.
Conclusion: MIRU molecular typing is a reliable and efficient method not only for epidemiological study and outbreak surveillance but also for determination of recurrence and reinfection of *Mycobacterium tuberculosis*. This method could also provide physicians with useful information for outcome treatment.

P1682 Diversilab typing for management of *Enterobacter cloacae* outbreaks at a neonatal ICU

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Objectives: In April 2007 the neonatal ICU observed a possible outbreak of *Enterobacter cloacae*. Concern was raised when several children presented with clinically significant infections due to *E. cloacae*.
Methods: To establish the relation between these infections all clinical isolates were collected and all patients were screened for the presence of *E. cloacae* in throat and rectal swabs. At approximately the same time a new typing system was introduced in our laboratory: Diversilab (bioMerieux). This system enables automated genotyping of bacteria, yeasts and moulds, applying kits for amplification of repetitive extragenic palindromic (REP-PCR) sequences. PCR fragments are analysed on an Agilent® 2100 Bioanalyzer. Web-based software stores sample fingerprints, normalizes the data for comparison and analyses similarity between fingerprints. To validate Diversilab typing, extracted DNA of all *E. cloacae* strains was also typed by AFLP as gold standard.
Results: Diversilab and AFLP data were concordant and showed identical strains in 5 patients and unrelated strains in 3 patients. Hygienic measures were enhanced in the following weeks and consequently the number of *E. cloacae*-positive patients decreased. In July a sudden increase in *E. cloacae*-positive cultures was observed, and we hypothesised that the outbreak strain persisted in a niche on the neonatal ward. Again strains collected from both surveillance and clinical cultures were typed with Diversilab and AFLP. Both typing systems showed that the hypothesis was refuted. An unrelated *E. cloacae* strain was responsible for the second outbreak. This strain was present in 5 patients. One patient, epidemiologically related to the first outbreak, tested positive for the outbreak strain of the first episode. One patient, who was present on the ward during both episodes, tested positive for an unrelated *E. cloacae* strain in both episodes. We concluded that both outbreaks on the neonatal ICU were not the result of one single persisting strain, but rather based on the successive introduction of unrelated *E. cloacae* strains. In October 2007 again an increase of *E. cloacae* positive cultures was observed and typing showed no new outbreak, as all but 2 strains were unrelated.
Conclusion: The analysis of the presented outbreaks demonstrated that the Diversilab is a very fast, robust and reproducible tool to determine clonal outbreaks of *E. cloacae* and typing of bacterial strains is crucial for adequate outbreak management.

P1683 PFGE analysis of MDR isolates of *Klebsiella pneumoniae* cultured from patients at Tehran hospitals

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Objectives: Multi-Drug Resistance (MDR) *Klebsiella pneumoniae* have caused major therapeutic problems worldwide due to emergence of Extended-spectrum β-lactamase (ESBL)-producing strains of *Klebsiella pneumoniae*. Identifying genetic phylogeny of hospital pathogens is

essential for finding source of infections and prescription of proper antibiotics are important for control of nosocomial infections.
The goals of this study were to determine the susceptibility of *Klebsiella pneumoniae* isolated from Tehran Hospitals to different antimicrobial agents and genetically characterize ESBLs isolates by pulsed field gel electrophoresis (PFGE).
Methods: 147 isolates of *Klebsiella pneumoniae* were collected from Tehran hospitals during September 2006 to July 2007, and tested for susceptibility to 16 antibiotics. Production of ESBL was assessed using Double Disk Synergy (DDS) and Phenotypic Confirmatory (PCT) Tests. The genomic DNA was extracted from the strains and separated by PFGE after digesting with Xba I endonuclease.
Results: Production of ESBL was detected in 72.8% of isolates. The majority of Neonatal and ICU isolates showed the ESBL phenotype. Susceptibility to imipenem and meropenem were 100% followed by levofloxacin (76.9%), piperacillin/tazobactam (53.7%) and ceftizoxime (58%) respectively. The most resistance was observed against ampicillin/sulbactam and aztreonam (71.4%), cefotaxime (66.7%) and gentamicin (62.3%).
In PFGE, all neonatal strains from one of the hospitals were identical. A dominant cluster was observed in ICU isolates of *K. pneumoniae*, too. But other isolates showed different genetic patterns.
Discussion: The most effective antibiotics against ESBL-producing K. pneumonia were carbapenems followed by levofloxacin. It appears that outbreaks with ESBL-producing *K. pneumoniae* had occurred in neonatal and ICU setting in one of the Hospitals.
PFGE is a reliable technique for genetic analysis of *K. pneumoniae* collected from community and it can be use to reveals the source outbreaks with MDR-*Klebsiella* pneumonia in hospitals.

P1684 Molecular characterisation of *Klebsiella pneumoniae* strains isolated from blood culture

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Klebsiella pneumoniae strains are recognised as a common cause of hospital acquired infections associated with bacteraemia and lethal sepsis.
Objectives: The aim of present study was to characterize the strains isolated from blood culture in hospitalised patients in order to detect a correlation between the genetic pattern of strains and the source of isolation.
Methods: A total of 31 strains isolated from blood culture of inpatients from four different hospital units for new born children were characterised by biochemical testing, phage typing and antimicrobial sensitivity testing. PCR targeting fur gene and other subtractive sequences noted by as SL002, SL003, SL019, SL020, SL021, and SL025 was used to identify the genetic pattern of strains. The genetic relatedness of the isolates was assessed by PFGE.
Results: All strains were PCR positive for fur gene and negative for SL021 and SL025 sequences. The most frequently detected sequences were SL020 (30 strains) and SL 019 (27 strains). SL002 sequence was identified in 6 strains and SL003 in 10 strains. When the total profile of amplification was analysed, the strains gathered in six different gene patterns. Two PCR profiles grouped 24 strains, the remaining 7 strains displaying other 4 gene profiles. When PFGE was used to characterize the strains a total of 11 patterns were identified. Strains displaying the same PCR profile showed the same PFGE type and suggested a common source of infection.
Conclusion: Strains isolated from the same hospital presented a correlation between phage type, PCR profile and PFGE pattern. When the phage typing could not discriminate between strains, PCR and PFGE were useful.
Similarity of PCR profiles and PFGE patterns suggests a hospital acquired infection.

P1685 Molecular epidemiology of plasmid-mediated AmpC β-lactamase and extended-spectrum-β-lactamase from clinical isolates of children in Wuhan area, China

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Objectives: To investigate molecular epidemiology characteristics of β-lactamases in plasmid-mediated AmpC and extended-spectrum-β-lactamases (ESBLs) producing in *Escherichia coli* and *Klebsiella pneumoniae* from children in Wuhan area.
Methods: A total of 228 clinic isolates, including 161 cefoxitin-resistant or medium, 67 cefoxitin-sensitive ESBLs producing *E. coli* and *Klebsiella pneumoniae* were collected at The Children's Hospital of Wuhan during a period from Aug 2005 to July 2006. Three-dimensional extract test was used for primary analysis of plasmid-mediated AmpC-lactamases and phenotypic confirmatory test was done for ESBLs. Transformation experiment was used to position drug resistant gene. Universal primer PCR for AmpC and CTX-M gene amplification and DNA sequencing were carried out for genotype of β-lactamases. Detection and subtyping of bla-CTX-M genes was done by Restriction Fragment Length Polymorphism (RFLP)analysis.
Results: Both DHA-1 and ACT-1 genotypes were determined to be the primary plasmid-mediated-AmpC-lactamase genes among the 228 isolates, and CTX-M-14 and CTX-M-3 genotype were proved to be the predominant ESBLs genes. The number of isolates producing both DHA-1 and ACT-1 at the same time was much higher than isolates producing only one kind of these AmpC enzymes. The combination genotypes of AmpC and CTX-M were observed in isolates. Among which, CTX-M-14+DHA-1+ACT-1 genotype was the much common isolates in *E. coli*. However, for *K. pneumoniae*, the main combination genotypes of AmpC and CTX-M was CTX-M-3+DHA-1+ACT-1. On the other hand, a new ACT genotype was founded in *Klebsiella pneumoniae*, and DNA sequence was shown that new ACT genotype has 84% homogeneity with ACT.
Conclusion: *E. coli* and *K. pneumoniae* producing both ESBLs genes and AmpC β-lactamase have higher proportions than that producing single plasmid-mediated AmpC enzyme. CTX-M-14 or CTX-M-3 plus DHA-1and ACT-1 was the primary genotype in children at Wuhan area.

P1686 Ribotyping in molecular epidemiological studies of uropathogenic *Escherichia coli*. A critical assessment

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Objectives: To compare PCR phylogrouping and ribotyping in epidemiological study of uropathogenic *E. coli*.
Methods: Fluroquinolone resistant (N=35) and sensitive (N=31) UTI isolates, commensal isolates (N=20) from Norway and UTI isolates from Russia (N=31) were investigated. Isolates were typed using PCR phylogrouping, Ribotyping and PFGE. Minimal inhibitory concentrations were determined for ampicillin, mecillinam, nitrofurantoin, sulphonamide, trimethoprim, tetracycline, nalidixic acid, ciprofloxacin, gentamicin and chloramphenicol. Virulence factors (type 1, P, and S fimbriae, papG class I, II and III, haemagglutinin, capsule K1, capsule K5, aerobactin and hemolysin) were detected by multiplex PCR.
Results: 33 ribotypes were found. 7 groups of similar ribotypes could be identified (Ribogroups RiboA–G). There was evident correlation between ribogroup and country of origin, biological source and fluoroquinolone resistance. Phylogroup correlated with ribotype and ribogroup but correspondence was not perfect. Ribogroup G was predominantly phylogroup D, urovirulent, and related to clonal group A (CgA). Antibiotic resistance (excepting fluoroquinolone) and virulence profiles were unrelated to ribogroup. Isolates with similar PFGE patterns had similar, but not always identical ribotypes.
Conclusion: Ribotyping is a convenient, but costly typing method that correlates with clinically relevant biological parameters and geographical origin. Surprisingly, ribotypes are not homogeneous with respect to

phylogenetic group, suggesting that the genetic loci tested may be subject to horizontal exchange, which might limit the value of these methods. The identification of a ribotype group, riboG, consisting of predominantly urovirulent Phylogroup D may provide insights into the origin of the urovirulent clonal group A (CgA).

P1687 Molecular typing of enteropathogenic *Escherichia coli* by multilocus sequence typing

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Objectives: This study analyses by MLST (Multilocus Sequence Typing) a collection of 30 enteropathogenic *Escherichia coli* (EPEC) isolated from patients with diarrhoea, admitted to an University Hospital from Brazil.
Methods: 30 strains of EPEC were studied and previously classified by different molecular techniques, including by pulsed-field gel electrophoresis. MLST was performed based on DNA sequences of 7 conserved housekeeping genes: adk (adenylate kinase), fumC (fumarate hydratase), icd (isocitrate dehydrogenase), purA (adenylosuccinate dehydrogenase), gyrB (DNA gyrase), recA (ATP/GTP binding motif) and mdh (malate dehydrogenase). The detailed protocol of the MLST procedure, including allelic type and sequence type methods, may be found at the MLST website. Clonal relationship among the 30 strains and the strains of MLST database was performed using eBURST V3 algorithm to obtain a population snapshot and to get the most likely patterns of evolutionary descent in our collection. An UPGMA dendrogram was constructed from the data matrix of allelic mismatches of the strains in this study, by START. To analyse the variable alleles, MEGA version 2.1 was used to construct a circle style phylogenetic tree, with the neighbour-joining algorithm and the nucleotide p-distance model of genetic distance.
Results: 6 new alleles were described which determined 14 new ST among 18 strains indicating the existence of considerable genetic variability among the strains; 20 strains were associated with 8 ST previously described. The strains were divided into 12 clonal complexes and CC10 was the major one, harboured 3 strains of this study and other 9 Brazilian strains, indicating an endemic prevalence of these clones in the country. The purA & recA housekeeping genes presented minor variation in relation to the other genes. The number of individual alleles for each one of the 7 housekeeping genes ranged from 8 for purA, 10 for recA, 11 for gyrB, 12 for adk, fumC, icd and mdh.
Conclusion: MLST has been rarely used in epidemiologic and phylogenetic studies involving EPEC strains, particularly in Brazil. There were only 23 strains in MLST *E. coli* database, and this study included other 30 strains of EPEC previously analysed and classified by different phenotypic and molecular techniques. This study allowed including *E. coli* data from Brazil in the *E. coli* MLST database and this fact permits the use of data about the Brazilian *E. coli* strains in epidemiologic worldwide studies.

P1688 Comparison between rep-PCR-based DNA fingerprint and sequence analyses of clinically relevant *Nocardia* species

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Objectives: Nocardiosis is a localised or disseminated infection caused by ubiquitous, soil borne, aerobic, and saprophytic actinomycetes. *Nocardia* species most often affect immunocompromised patients. *Nocardia* asteroides (causes at least 50% of invasive infections). Other pathogenic species include *N. farcinica*, *N. nova*, *N. transvalensis*, *N. brasiliensis*, and *N. pseudobrasiliensis*. In the United States, an estimated 500−1,000 new cases of *Nocardia* infection occur annually and other reports indicate fewer instances in European countries and Japan. Generally, for accurate identification to the species level sequencing is performed. rep-PCR based DNA fingerprinting has shown promise for rapid species and strain-level discrimination for some bacteria and fungi. In this study, we compared rep-PCR-based DNA fingerprint and sequence analyses of clinically relevant *Nocardia* species.
Methods: A total of 12 *Nocardia* species were purchased from ATCC including *N. abscessus*, *N. africana*, *N. asteroides*, *N. brasiliensis*, *N. brevicatena*, *N. carnea*, *N. farcinica*, *N. nova*, *N. otitidiscaviarum*, *N. paucivorans*, *N. pseudobrasiliensis*, and *N. transvalensis*. In addition, 30 clinical isolates were collected and analysed. The isolates were cultured, and genomic DNA was extracted from each isolate. DNA was then amplified using the DiversiLab *Nocardia* Fingerprinting Kit (beta version). The data were processed using the DiversiLab software.
Results: DNA fingerprints were obtained for all *Nocardia* species using the DiversiLab System. Rep-PCR-based clustering of all *Nocardia* isolates was in agreement with the sequence-based identification of the clinical isolates and the ATCC isolates. Additionally, the rep-PCR method showed sub-species discrimination.
Conclusions: The DiversiLab System is a rapid molecular genotyping tool that shows promise for differentiation of *Nocardia* species. The sub-species discrimination indicates potential as a strain typing tool for epidemiological studies.

P1689 Molecular diversity among Bulgarian *Bacillus anthracis* strains: the emergence of novel genotypes

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Objectives: Human anthrax is a relatively rare disease in Bulgaria with up to ten cases per year occurring mainly in two endemic regions located in the Northern part of country. After the bioterrorism attacks in 2001 a particular interest has emerged for introducing efficient high-resolution typing systems that are readily applied for the global mapping of the *B. anthracis* genotypes. The current study was conducted in order to investigate the molecular diversity among a Bulgarian collection of *B. anthracis* isolates collected for more than 50 years and to shed some light on the genotypes circulating on the Balkans.
Methods: A total of 43 *B. anthracis* isolates positive for both virulence plasmids pXO1 and pXO2 were included in the study. The isolates were previously cultured either from humans, animals or from environmental sources for the period 1960–2006. They were identified by several different conventional methods as well as by specific PCR that was targeted at two chromosomal and two plasmid markers. The multilocus variable number of tandem repeat analysis (MLVA) system proposed by Keim et al. was applied for studying the molecular diversity among these isolates. The fragment analysis was carried out in a fully automated high-resolution capillary system HDA-12 (eGene Inc., USA) which produces results within six minutes.
Results: The isolates were grouped in three major clusters with several subclusters. The chromosomal vrrC1 and vrrC2 markers were characterised by two previously undescribed alleles with sizes 436 bp and 334 bp respectively found in seven strains each and generating a total of four novel genotypes. These new alleles found so far only in Bulgarian isolates were also verified by sequence analysis. Interestingly one of the new genotypes was associated with a fatal case of septic anthrax. More than half of the investigated isolates (n = 23) were grouped within the A1.a subcluster (Keim et al.) which ranges over a geographically heterogeneous group with members from Europe and North America.
Conclusions: The study brings information on the molecular diversity of *B. anthracis* on the Balkans and reveals several previously undescribed genotypes among the Bulgarian isolates. The MLVA8 typing system represents an efficient tool for studying the epidemiology and phylogenetics of this high-risk pathogen.

P1690 A common F. tularensis outbreak MLVA genotype in the Balkans

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Objectives: Tularaemia is a major problem in the Balkans and in Bulgaria. After publishing our initial results for Bulgaria, strains from Turkey were obtained and comparative genetic analysis realised.

Methods: In this study we have performed high resolution multiple-locus variable-number tandem repeat analysis (MLVA) in order to compare the Bulgarian and Turkish strains from the two outbreaks and to shed some light on the genotypic structure of F. tularensis populations on the Balkans and Anatolia.

Results: A total of four genotypes were found among the nine isolates investigated (7 Bulgarian and 2 Turkish). The M3 marker appeared to be the most variable one showing four different alleles among the nine strains studied followed by M6 (3 alleles) and M20 (2 alleles). All other markers were presented with a single allele. The two Turkish strains were found to be identical to each other and identical to one of the Bulgarian outbreak clusters.

Conclusion: Using the MLVA method we obtained a direct evidence for identity of strains isolated in Turkey and Bulgaria. As the two outbreaks occurred in very distantly located regions (over 500 km) a direct epidemiological relation is highly improbable however a common Balkan genotype could exist.

P1691 Evolution of endemic clones of *Legionella pneumophila* serogroup 1 in France from 1995 to 2007

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Objective: In France, *Legionella pneumophila* serogroup 1 (Lp1) is involved in more than 80% of Legionnaires' disease cases. This infection can be due to sporadic (unrelated isolates with unique genotype), epidemic (same genotype shared by time and space related isolates) or endemic (same genotype shared by epidemiologically unrelated isolates) strains of *Legionella*. The aim of this work was to monitor the evolution of the populations of Lp1 endemic strains isolated from clinical samples in France from 1995 to 2007.

Methods: All French *Legionella* clinical isolates were systematically genotyped by pulsed-field gel electrophoresis (PFGE). The French database of *Legionella* PFGE patterns was analysed (~2000 patterns of clinical strains isolated from 1995 to 2007). The sequence types (ST) of unrelated isolates sharing identical PFGE patterns were determined.

Results: Five different populations of unrelated isolates with same genotype (PFGE patterns and ST) were identified; these populations belonged to the five major French endemic clones: Paris, Lorraine, Louisa, Mondial and Biarritz. Their distribution over the time was different: (i) the Paris strain was the major endemic clone identified (its isolation rate ranged from 6 to 12% since 1996); (ii) the Lorraine strain has emerged since 2002 (it has accounted for 10% of clinical isolates since 2005); (iii) the Louisa strain was firstly detected in 2001 and has emerged since 2004 (from this year, its clinical isolation rate has increased continually reaching to 7% in 2007); (iv) the Mondial strain was identified in 1998 during an outbreak (from this year, its clinical isolation rate has decreased progressively); (v) the Biarritz strain was detected almost every year with an isolation rate never higher than 5%. In total, these 5 endemic clones accounted for up to 29% of clinical isolates in 2006 and 2007.

Conclusion: Five major endemic clones of Lp1 have been identified, in France. The distribution over the time of these clones, isolated from clinical samples, was variable. Some clones seemed to be continually detected, at a low level (Biarritz) or at a higher level (Paris). The Mondial clone seemed to disappear with the year, whereas the Lorraine and the Louisa clones seemed to emerge among the clinical isolates for the past few years.

P1692 Cluster of human listeriosis cases traced by molecular typing to taleggio cheese, Italy

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Objectives: *Listeria monocytogenes* is a foodborne pathogen responsible for severe invasive disease in humans and animals. A number of large outbreaks have been described in many industrialised countries, but most cases are considered to be sporadic.

Frequent clustering was detected by a retrospective subtyping analysis of 24 human isolates identified from apparently sporadic cases of infection in the Lombardy region, Italy, in the years 2006–2007, by using serotyping and DNA-based subtyping methods. The objective of this study was to evaluate a possible causative role of *L. monocytogenes* strains isolated from cheese and salami samples on sale in the same geographic area.

Methods: Serotyping

All strains tested were serotyped following the manufacturer's instructions using commercial specific antisera (Denka Seiken, Tokyo, Japan).

Ribotyping

Isolates were characterised by automated ribotyping using the RiboPrinter® (Qualicon, Inc. Wilmington, DE, USA) and selecting EcoRI as the restriction enzyme. Ribotypes were firstly classified into ribotypes by automatic classification and, then, some isolates were submitted to manual classification after visual inspection.

PFGE analysis

PFGE was performed according to PulseNet protocol with enzymes AscI and ApaI. New PFGE profiles obtained by AscI were marked by subsequent numbers. Closely related patterns, differing from each other by one to three bands, were assigned an additional capital letter. Indistinguishable or closely related strains were subsequently cleaved with ApaI.

Results: Seven clusters including two to three isolates were identified within human isolates. Isolates from salami were heterogeneous but all were different by ribotyping and PFGE from the available human isolates. Taleggio and gorgonzola cheese samples proved to contain two different strains of *L. monocytogenes*.

A cluster, including human isolates from the geographic area under study, consisted of three strains indistinguishable by serotyping – serotype 1/2b, PFGE after AscI and ApaI digestion and EcoRI ribotyping – ribotype DUP-1034 – from *L. monocytogenes* strains detected on the rinds of Taleggio cheeses produced in an Italian plant.

Conclusion: Listeriosis control strategies should include subtyping of human isolates and take into account that a large proportion of cases may represent foodborne outbreaks. PFGE and ribotyping databases representing isolates from different sources may be invaluable to identify source-specific subtypes.

P1693 Relapsing cellulitis and bacteraemia due to *Campylobacter coli* in an agammaglobulinemic patient

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Objective: *Campylobacter* spp., a frequent aetiologic agent of diarrhoea in humans worldwide, uncommonly causes bacteraemia or extraintestinal infections especially in immunocompromised patients.

Aim of this study is to describe a case with X-linked agammaglobulinaemia (XLA) with relapsing episodes of leg cellulitis and bacteraemia due to *C. coli*. The work aims also to characterize, by molecular methods, all patient's *C. coli* isolates including a strain isolated from the patient's stool in 1997.

Methods: Stool, blood and skin lesion specimens were collected and cultured as conventional protocols. Identification of isolates was performed by biochemical and PCR tests as previously described. Susceptibility to erythromycin, gentamycin, kanamycin, nalidixic acid, ciprofloxacin, norfloxacin, ampicillin, amikacin, cephalotin, cefotaxime,

ceftazidime, ceftriaxone, cefoxitin, imipenem, cloramphenicol, trimethoprim/sulphametoxazole, tetracycline, was tested by disk diffusion method.
Molecular characterisation of strains was performed by Pulsed Field Gel Electrophoresis, using SmaI and SalI as restriction enzymes; Multi Locus Sequence Typing were also applied to all isolates. PCR and sequencing were performed to detect mutations within 23S rDNA gene, conferring erythromycin resistance.
Results: Stool, blood and skin lesion culture were positive for *C. coli*. From stools 2 colonies with different morphology (A, B) were both identified as *C. coli*. All strains were susceptible to imipenem, cloramphenicol, tetracycline, gentamycin, kanamycin, amikacin. The strain from colony A was also susceptible to erythromycin. All strains were resistant to quinolones, fluoroquinolones and β-lactam antimicrobials. Oral tetracycline associated with bacitracin/neomycin was then administered. Stool, blood, skin lesion cultures became rapidly negative. After 3 weeks tetracycline therapy was discontinued and he was treated for additional four weeks with bacitracin/neomicyn. All cultures were persistently negative and there has been no disease recurrence after 3 months.
Pulsed Field Gel Electrophoresis performed with both enzymes and analysis by Multi Locus Sequence Typing showed genetic identity for all *C. coli* strains including the 1997 isolate.
Conclusions: This is the first isolation of *C. coli* from cellulitis lesion. In spite of *Campylobacter* genetic instability, all isolates from this patient were genetically identical supporting a latent infection in the intestine for years.

P1694 Molecular characterisation of *Campylobacter jejuni* isolated from healthy bovine, ovine and free-range poultry faeces in the Basque Country (northern Spain)

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Objectives: Domestic farm animals are reservoirs of pathogenic campylobacters like *Campylobacter jejuni*, the most frequently isolated bacterial pathogen in cases of human gastroenteritis in developed countries. A prevalence study carried out in the Basque Country identified 28.3% (34/120) of ovine and 18.0% (37/206) of bovine farms positive for *C. jejuni*, and even higher values (38.2%, 13/34) in free-range poultry farms. The high incidence of human *Campylobacter* infections in the Basque Country (121 cases per 100,000 inhabitants in 2005) and the relatively high prevalence in primary production units of this foodborne pathogen prompted us to further characterize the animal isolates to investigate the relationships among strains from different sources.
Methods: A selection of isolates from the different host species (sheep, cattle and poultry) were analysed using three molecular typing techniques: pulsed-field gel electrophoresis (PFGE) of pure cultures digested with SmaI, restriction fragment length polymorphism analysis of the PCR amplified flaA gene (flaA PCR-RFLP) and multilocus sequence typing (MLST) of seven housekeeping genes (aspA, glnA, glyA, gltA, pgm, uncA and tkt).
Results: PFGE and MLST showed higher discriminatory power than flaA PCR-RFLP since flaA PCR-RFLP profiles present in different production systems were further discriminated by both PFGE and MLST. Nevertheless, different genetic types were observed between farms and within the same farm suggesting the co-existence of several strains probably originating from different sources.
Conclusion: The use of at least two molecular typing methods is needed for optimal discrimination. Typing of *C. jejuni* strains was a useful tool for the identification of sources of infection within the farm. In addition, MLST data provided information on the overall population structure of the organisms that can be used in future population genetic approaches.

P1695 Genotyping *Helicobacter pylori* from faecal DNA

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Objectives: *Helicobacter pylori* is the commonest chronic bacterial infection of mankind. It is usually acquired during childhood and in the developed world it is the principal cause of duodenal ulcer disease and a risk factor for gastric cancer. In the developing world it is associated with undernutrition and short stature. Non-invasive methods of diagnosis and detection involve the urea breath test, serology and faecal antigen analysis. However genotyping bacterial isolates from children could enhance epidemiological investigations into routes of transmission of this microorganism. This study exploits the excretion of *H. pylori* in the faeces to obtain *H. pylori* DNA for genotyping and thereby to generate dendograms to explore patterns of intrafamilial transmission.
Methods: Subjects were children (<16y) attending the Royal Hospital for Sick Children in Glasgow for urea breath test for suspected *H. pylori* infection. Each subject, and close family members (parents and sibs), was asked to provide a stool sample, which was stored at −80ºC until analysis. Twenty-eight stool samples that were positive by HpSA faecal antigen test (Meridian Diagnostics Inc) were analysed. *H. pylori* DNA was recovered using phenol: chloroform: isoamylalcohol and ethanol precipitation followed by isolation using a specific biotinylated oligonucleotide probe with magnetic capture. RAPD PCR [1] was used as a means of genotyping *H. pylori* DNA isolated from faeces.
Results: Data from sixteen families were analysed. One family had four members, one had three members, seven had two members and seven had one member. RAPD analysis revealed a typeability of ninety-three percent. Loose similarities were observed between family one involving mother and children but not between father and children. Similarly in two other families samples from mother and child bore similarities. All parent samples and those from other families bore little correlation.
Conclusion: Isolation of purified *H. pylori* by gene capture offers a means of extracting DNA from faecal samples which can be use to genotype the microorganism. The majority of faecal *H. pylori* isolates were successfully genotyped using RAPD which proved to be highly discriminating.

Reference(s)

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P1696 Is *Helicobacter pylori* the only aetiologic factor involved in the pathogenesis of gastric cancer among Iranian population?

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Objectives: *Helicobacter pylori* has been documented as an aetiological agent in gastric cancer (GC) development. Hp immuno-reactive proteins and its main virulence factors, CagA and VacA, are implicated in the pathogenesis of related diseases. On the other hand, host genetic susceptibility and gastric hormones have been proposed as important indicators in screening approaches for GC development. This study has aimed to explore the aetiologic role of Hp, its antigens and their association with the other mentioned factors in developing GC among Iranian patients.
Methods: Hp status was determined by three gold standard tests. 309 patients referring for endoscopy and 198 GC cases were included in this study. Immunoblotting, ELISA and gene-specific PCRs were performed on collected samples.
Results: Hp infection was detected in 78.8% of all subjects composed of 80.3% of dyspeptic (non GC) subjects and 76.5% of GC patients. Seroreactivity toward Hp was detected in 91–93% of both studied groups. vacA s1m1 and s2m2 genotypes were most prevalent in GC and non GC patients, respectively. Serological data revealed that unlike cagA genotyping which revealed no association with disease, the presence of anti CagA antibody can be a predictive marker in GC development. Antibody response towards Hp LMW antigens especially 35kDa and 37kDa proteins are differentially prevalent in two studied groups. Histopathological data revealed that there is no significant association between

the type of tumour and Hp positivity. Host interleukin-1 genotyping indicated that there is a higher prevalence of Hp infection in subjects with L/L genotype of IL-1RN irrespective of the clinical picture while Hp negative subjects possess mostly the 2/2 allele. In both Hp positive and negative groups the mean and standard deviation of pepsinogen I and II levels were similar.
Conclusion: This study showed that although the absolute majority of the studied subjects were infected with Hp, genotyping of isolated Hp strains and serological analysis can be appropriate screening approaches for high risk populations. Evaluation of host susceptibility factors revealed that IL-1 beta and its receptor antagonist may be involved in GC pathogenesis irrespective of Hp infection. The genetic background of the normal population, however, should be investigated before making a firm conclusion about this contribution to GC development.

P1697 Human papillomavirus genotyping before the introduction of quadrivalent vaccine against HPV on the Romanian population

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Objective: To study the prevalence of Human Papillomavirus (HPV) genotypes in Romanian population before the introduction of anti HPV vaccine in order to be able to find out the changes that will occur in HPV genotypes in our population once the vaccine will be applied.
Methods: 115 patients, average age 32.8±8.85 years (limits 3–63 years old), 82% women, were tested for the presence and genotype of HPV during a period of one year before the introduction of HPV vaccine. The patients presented with cervical lesions, vulvar, vaginal, or penian warts, cervical intraepithelial neoplasia grade 2 or 3, adenocarcinoma in situ, conjunctival papillomas, oral papillomas, and other cutaneous lesions and as sexual partners of women diagnosed with HPV. Genotyping was performed after the amplification of the extracted DNA target by hybridisation on strips provided through Inno-Lipa HPV Genotyping CE Assay.
Results: We found a high prevalence of the HPV infection in the studied population. 79.3% patients were HPV positive, but women were more affected than men: 88.8% positive women vs. 63.6% positive men. The following genotypes were founded: HPV 6 (in 17.4% cases); HPV 16 (in 17.4% cases); HPV 18 (in 8.7% cases); HPV 31 (in 21.7% cases); HPV 51 (in 13% cases); HPV 53 (in 4.3% cases); HPV 66 (in 4.3% cases); HPV 68 (in 4.3% cases); HPV 70 (in 4.3% cases) and in 26% cases we could not determine the genotype. Also 17.4% from the positive patients presented at least 2 HPV genotypes at the same time. The most frequent associations were: 6 + 68; 16 +18 + 31; 18 + 31 and 16 + 51.
Conclusion: Due to the high prevalence of HPV infection in our population we recommend molecular diagnostics and genotyping as a routine examination and a possible indication for the anti HPV vaccine in patients with characteristic lesions for HPV infection.

P1698 A novel DHPLC assay for identifying genotypes of SHV-type

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Objective: To develop a sensitive, rapid and high-throughput method using DHPLC (Wave® Nucleic-Acid Fragment Analysis System, Transgenomic) for genotyping blaSHV producing ESBLs.
Method: PCR primers were designed to amplify 234bp fragments of the ORF of all blaSHV. Six well-defined blaSHV ESBL-producing strains (blaSHV-12, -2a, -6, -14, -55 and -5) were used as controls to develop and optimize the DHPLC genotyping assay. The blaSHV-11 strain was used as the reference strain. Heteroduplex PCR products were formed by mixing an equal amount of DNA (5µl) amplified from the reference strain blaSHV-11 and one of six blaSHV control strains. The mixtures were heated to 95ºC for 5 min and then gradually cooled to 25ºC to form heteroduplex DNA molecules. Aliquots of 6 µl duplexed PCR products were then analysed by DHPLC on the Wave® Nucleic-Acid Fragment Analysis System.
Results: The sequences of the PCR amplicons of the reference and control strains were aligned and analysed. It was found that two substitutions led to a change in the amino acid sequence and four were silent mutations. Unique DHPLC chromatogram profiles were obtained for all six blaSHV types.
Conclusion: This is a first report of a novel DHPLC assay for genotyping of blaSHV ESBLs; it offers a specific, sensitive, economical and high-throughput approach to screen blaSHV producing ESBLs. The two most frequently encountered genotypes worldwide, blaSHV-12 and -5, were easily distinguished from each other and other genotypes tested. It has a great potential for determining the clinical relevance of different known and new blaSHV genotypes, as well as for epidemiological studies and surveillance programmes. Applying the newly developed dHPLC assay for genotyping blaSHV positive clinical isolates is currently being undertaken.

P1699 Phylogenetic analysis of a rubella outbreak in Madrid, Spain in 2004/2005

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Objective: Phylogenetic analysis of an rubella virus outbreak from Madrid, Spain in 2004/2005.
Methods: Serum, oropharyngeal swab and urine specimens were collected from patients from a Rubella outbreak which occurred in 2005 in the Community of Madrid. The outbreak affected mostly Latin American immigrant population and Spanish men. The isolation was realised in Cell Culture and confirmed by immunofluorescence assay and nested reverse transcription-PCR. Phylogenetic analysis of the 739 nucleotides from the E1 gene recommended by Word Health Organization (WHO) was realised in all isolated and clinical samples using MEGA v3.1 software.
Results: Eighteen isolates out of 38 clinical samples from the outbreak were obtained, including two isolated from congenital rubella syndrome (CRS) cases. These results showed that only one RUBV genotype circulated in Madrid during the outbreak, and it was assigned to genotype 1j. The homology range falls into 97.8 to 98.2 compared with the reference sequence of genotype 1j.
Conclusion: Probably, the origin of this genotype was not Latin American, because the circulation in that region corresponded to genotype 1C. Moreover, the published sequences of genotype 1j come from Japan and Philippines. Finally, this study also represents the first report of detection of this genotype in Europe.

P1700 Multilocus sequence typing for *Candida albicans* strains from patients with candidaemia

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Objectives: To investigate the clonal stabilities or evolutionary changes of *C. albicans* isolates from candidaemia patients, and to investigate whether the candidaemia agents diagnosed at our hospital are related to internationally distributed *C. albicans* lineages, we performed multilocus sequence typing (MLST) of 45 *C. albicans* isolates from various clinical specimens of 23 patients with candidaemia.
Methods: The internal regions of seven housekeeping genes (AAT1a, ACC1, ADP1, MPIb, SYA1, VPS13, and ZWF1b) were sequenced. The MLST results were compared with the results of Southern blot hybridisation of the C1 fragment of Ca3 as the probe (C1 fingerprinting) and pulsed-field gel electrophoresis (PFGE) analysis. PFGE analysis included restriction endonuclease analysis of genomic DNAs using BssHII (REAG-B) and SfiI (REAG-S).
Results: Overall, the 45 isolates yielded 20 unique diploid sequence types (DSTs) by MLST, 19 different REAG-B patterns, 18 REAG-S patterns, and 21 C1 fingerprinting types. By MLST, 33 isolates from 18 patients belonged to new sequence types (16 distinct DSTs), whereas 12 isolates from 5 patients belonged to the previously described types (4

DSTs). The intra-individual isolates showed minor genetic differences (microevolution) by MLST (5 patients), REAG-B (4 patients), REAG-S (2 patients), and C1 fingerprinting (4 patients). All of four genotyping methods used revealed that two distinct genotypes were shared among 10 isolates from nine patients in a neonatal intensive care unit, suggesting two nosocomial clusters. However, none of the blood isolates from these two clusters showed any microevolution by any of the genotyping methods used.

Conclusion: The present study shows that MLST had similar or greater discriminatory ability than PFGE or C1 fingerprinting, and some of the *C. albicans* strains that cause candidaemia at our hospital share the same MLST type with strains from other geographic regions of the world. In addition, a genetically stable strain of the *C. albicans* strains may be responsible for nosocomial cross-infection.

P1701 25S intron-restriction endonuclease genotyping of *Candida albicans* isolates

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Objectives: There isn't any universally accepted gold-standard genotyping method for differentiating *Candida albicans* isolates. The presence of transposable group I intron in the 25S rDNA gene makes *C. albicans* isolates divided into three genotypes by 25S intron analysis. Genotype-A isolates were previously shown to contain different subgroups which were determined by restriction endonuclease and sequence analysis. In this study, our aim was to establish a new genotyping method based on PCR amplification of 25S intron region followed by restriction endonuclease analysis with two different endonucleases.

Methods: 111 *C. albicans* strains obtained from different infection sites of 64 leukemic patients were genotyped. PCR amplification was performed by using primers designed to span the transposable group I intron region of the 25S rDNA gene. To obtain the genotypes, PCR products were digested by HaeIII and MspI restriction endonucleases. The resulting band patterns were determined by agarose gel electrophoresis. Groups were differentiated due to the differences in the patterns. Discriminatory power (DP) was calculated for each method.

Results: 25S intron analysis divided the isolates into three genotypes. Eightyone of the isolates were found to be genotype-A, 18 genotype-B, and 12 genotype-C (DP= 0.43). HaeIII restriction of genotype-A isolates gave 18 subgroups, while genotype B and genotype-C isolates clustered in single subgroups (DP = 0.89). MspI restriction of genotype A isolates gave 14 subgroups, while genotype-B and genotype-C isolates again clustered in single subgroups (DP = 0.80). When the results obtained by both enzyme digestions were combined, 33 subgroups were obtained for genotype-A isolates, while genotype-B and genotype-C isolates both gave single subgroups (DP = 0.92).

Conclusion: As the DP of 25S intron analysis is below the acceptable limits (<0.90), its use for investigating *C. albicans* strains epidemiologically would not be appropriate. By combining 25S intron genotyping with HaeIII and MspI restriction endonucelase analyses, the DP rises to 0.92 and it becomes suitable for epidemiological purposes. The method is easy to perform, cheap, and labour firendly.

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P1702 ompA genotyping and epidemiological investigation of *Chlamydia trachomatis* urethritis in male patients, Athens, Greece

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Objectives: The epidemiological investigation of male urethral infections due to *Chlamydia trachomatis* in Athens, Greece.

Methods: During the period 2005–2006, a total of 210 male patients with acute urethritis, referred to the Outpatient Dpt. of the "Andreas Sygros" Hospital for Skin and Venereal Diseases, were enrolled in the study. Diagnosis of *C. trachomatis* and *Neisseria gonorrhoeae* was performed on intra-urethral swabs using conventional microbiology and the CT/NG COBAS AMPLICOR system (Roche Inc, Branchburg, USA). Chlamydial serotype assignment was performed after amplification and DNA sequencing of the whole *C. trachomatis* ompA gene. Patient information collected included age, profession, nationality, place of residence, family status, antibiotic use during the previous three months, sexual preference and number of sexual companions during the previous six months, other sexually transmitted disease in the past, and duration from symptoms onset to hospital visit. Statistical analysis was performed using the SPSS 13.0 software.

Results: In total, 46 of the 210 (21.9%) patients tested were diagnosed with *C. trachomatis* urethritis.

Serotypes E (34.8%) and G (26.1%) prevailed; additional serotypes recorded were F (13.0%), D (13.0%), Ja (10.8%), and B (4.3%). Overall gonococcal co-infection rate was 28%. Among patients with serotype Ja infection, co-infection rate was 100%, whereas the respective rates for serotypes D, E, F and G, were 50, 19, 16, and 16% (p = 0.003). Mean patient age was 31 years (range 16–57). Patients with a serotype D infection had a mean age of 36 years (range 22–49), whereas patients with an infection due to other serotypes had a mean age of 29 years (range 16–57). Mean duration to hospital visit was 17 days (range 1–95 days). Patients with an infection due to serotype Ja presented earlier to the hospital (mean duration to visit 3 days), whereas for serotypes G, B, E, D and F, time to visit were 10, 12, 20, 23, and 33 days respectively (p= 0.077 and 0.058 for comparing mean durations for serotypes D, E and F with those for G or Ja, respectively). Mean number of sexual companions was 4 (range 1–24).

Conclusion: In this first report of epidemiological investigation of *C. trachomatis* in Athens, Greece, serotypes E and G prevailed among male patients diagnosed with acute urethritis. Serotype distribution was associated with gonococcal co-infection rates and duration from symptoms onset to hospital visit.

P1703 New tests for syphilis: rational design of a PCR method for detection of *Treponema pallidum* in clinical specimens

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Objectives: A sensitive and specific PCR method to detect *Treponema pallidum* in clinical specimens was developed. PCR primers were designed based on two unique features of the DNA polymerase I gene (polA). The first distinctive characteristic is that the region codes for a high cysteine content and has low homology with similar regions of DNA polymerase I gene from known microorganisms. The second unique feature is the presence of four insertions in the gene. PCR tests using primers designed on the basis these regions reacted with various pathogenic *T. pallidum* subspecies but did not react with nonpathogenic treponemal species or other spirochetes. An additional 59 species of bacteria and viruses, including those that cause genital ulcers, tested negative. This PCR method is extremely robust and sensitive. The detection limit is about 10 to 25 organisms when analysed on gel. However, the analytic sensitivity can be increased by at least 1 log, to a detection limit of a single organism, when the ABI 310 Prism Genetic Analyzer is used to detect fluorescence-labeled amplicons. We further used this test in a clinical setting and compared the results with results from a previously reported multiplex-PCR test (for *T. pallidum*).

Results: We tested 42 genital ulcer specimens by the polA PCR, obtaining a sensitivity of 95.8% and a specificity of 95.7%.

Conclusion: These results suggest that the polA PCR is applicable as a routine clinical diagnostic test for syphilis.

P1704 **Sequencing and molecular typing of hypervariable loci in the *Treponema pallidum* genomes: characterisations of type strains and clinical isolates**

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Objectives: Using Comparative Genomic Sequencing (CGS) approach, hypervariable regions in *Treponema pallidum* genome were identified. These regions comprised hypothetical genes TP0136 and TP0548. Nucleotide sequences of these genes were determined and compared in 4 groups of *T. pallidum* strains: in ssp. *pallidum* (6 strains causing syphilis), in ssp. *pertenue* (6 strains causing yaws), in ssp. *endemicum* (2 strains causing endemic syphilis) and in 1 strain of unclassified simian isolate. In addition, 9 PCR positive clinical samples were analysed.
Methods: Primary screening of clinical specimens was performed using nested PCR detection of two *T. pallidum* specific loci (tmpC, polA). In PCR positive samples, direct Sanger sequencing of TP0136 and TP0548 PCR products was used to determine the corresponding sequences. Sequencing reads were analysed using computer-assisted assembly and the final nucleotide and protein sequences were clustered.
Results: The TP0136 and TP0548 analysed loci showed discrete clusters in 4 groups of type strains. Analysis of strains belonging to ssp. *pallidum* revealed two discrete subclusters containing Nichols-like and SS14-like sequences, respectively. All clinical isolates clustered with the SS14-like subgroup. In 4 clinical isolates, unique sequences in either TP0136 or TP0548 were found. Sequencing of different samples obtained from the same patient resulted in identical sequences.
Conclusions: Our results showed that TP0136 and TP0548 loci represent promising targets for molecular typing of pathogenic treponemes including clinical samples.
This work was supported by the grants from the Internal Grant Agency of the Ministry of Health of the Czech Republic (NR/8967–4/2006) and from the Grant Agency of the Czech Republic (310/07/0321).

P1705 **Genotyping of *Giardia lamblia* by multilocus sequence typing**

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The intestinal parasite *G. lamblia* occurs worldwide and infects humans and animals. The infection route is expected to be the oral–faecal route between humans, and between humans and animals. To be able to trace the sources of infection and to elucidate the zoonotic potential of *G. lamblia*, a high resolution genotyping method is needed to trace individual strains of *G. lamblia*.
Objective: Development of a multilocus sequencing typing method (MLST) for *G. lamblia*.
Methods: The MLST uses sequence variation in the genes for ferredoxin (FD), glutamate dehydrogenase (GDH) and giardin (GD) to make a genetic fingerprint of *G. lamblia* strains. The method can be used on faeces from patients without the need to culture this fastidious parasite.
Results: The MLST was validated on a panel of hundred patients with well described medical and demographic data. The faeces samples were collected in the routine diagnostics during a three month period in the western urban area of the Netherlands. In a previous study the two genotypes assemblage A and B were determined of the *G. lamblia* strains of these patients. We found thusfar 9, 8, and 11 variants of the FD, GDH, and GD gene respectively. In the patient samples we have analysed 28 samples yet and found eight different MLST types, indicating that the method has a good discriminatory power. The MLST types were grouped by cluster analysis. As expected, there was a clear digotomy between the MLST types of *G. lamblia* strains with A or B assemblages of these patients, indicating the large genetic differences between these two genotypes. Moreover a clear correlation between MLST type and demographic data of the patients was found, indicating that the method is able to distinguish epidemiologically related groups.
Conclusions: The described MLST using three genes is able to distinguish *G. lamblia* strains from separate outbreaks. Currently, we are working on the implementation of two additional genes in the MLST sceme to increase the resolution of this genotyping method.

Epidemiology of Gram-positive bacteria including pneumoccoci

P1706 **Trends in incidence of and serotypes causing invasive pneumococcal disease in Finland during 1995–2007: implications for introduction of conjugate vaccine**

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Introduction: Pneumococcal (Pnc) conjugate vaccines (PCV) decrease vaccine type specific incidence of invasive Pnc disease (IPD) but also increase non-vaccine serotype IPD. How much such phenomena is caused by underlying secular trends and evolutionary processes irrespective of PCV use is not well known. In anticipation of PCV introduction into national immunisation programme, we analysed trends of IPD and causative serotypes in Finland during years 1995–2006.
Methods: Notifiable IPD was defined as clinical condition with Pnc isolated from normally sterile site (blood, CSF, other). Isolates were obtained from adults and children hospitalised for IPD. Pnc were serotyped using quellung, Latex and/or CIEP (antisera from StatensSerumInstitute, Denmark) in KTL Reference Laboratory.
Results: Total 7193 Pnc isolates were identified. During observation period, overall IPD incidence grew significantly (1.5-fold) from 9/100 000 in 1995 to 14/100 000 in 2006. Increase was significant in children <5 and adults >50 years. Strong seasonal pattern in IPD was observed with annual peaks December and May. In 2006, most common Pnc serotypes were 14, 4, 6B, 23F, 3, 7F, 9V and 19F constituting 70% of all isolates, although among children <5 serotypes 3, 4, 7F, and 9V were observed less frequently. Since 1995, proportion of serotypes 14 and 9V had significantly increased, 4 and 7F decreased, while proportion of others remained constant, as did composition of most prevalent serotypes in children <5 (i.e. 6B, 14, 19F, 23F, 18C, 19A). During observation period among children <2 years, coverage of 7PCV serotypes fluctuated between 47 and 81% (mean 70%). Among those >2 years, coverage of 7PCV serotypes increased significantly from 47 to 59% (p for trend 0.000).

Age group	7PCV	10PCV	13PCV
<2 years	47–81; 70	55–84; 75	78–96; 91
	NS	NS	NS
⩾2 years	47–59; 53	57–66; 62	73–80; 77
	0.000	0.03	NS

In Table, range and mean % of vaccine serotype coverage among those to be vaccinated (<2 year olds) and those who might benefit from herd effect of PCV (>2 year olds), and p for linear trend of increase during 12 years of observation period.
While incidence of IPD has increased in past 12 years, seasonal pattern and constancy in composition of majority serotypes prevails with fluctuation in rank order of prevalent serotypes among children <2 years. In others, proportion of 7PCV and 10PCV serotypes has significantly increased.

P1707 ***Streptococcus pneumoniae* septicaemia associated with red cell transfusion**

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Background: Bacterial contamination of blood components remains an important residual infective risk of transfusion. We report a case of red

cell (RC) transfusion-transmitted bacterial infection (TTBI) caused by *Streptococcus pneumoniae*. This fragile non-commensal organism has not to our knowledge previously been implicated as a cause of RC TTBI.
Case and Investigation: The transfusion recipient was a 79 year old man with a myelodysplastic syndrome pancytopenia, with no history of splenectomy. Forty minutes after commencement of a RC transfusion he became febrile to 39.6ºC, hypotensive and hypoxic. Transfusion was ceased, and empiric therapy with intravenous antibiotics instituted with good clinical effect.
Cultures performed on recipient samples and the RC each demonstrated heavy growth of Gram-positive alpha-haemolytic diplococci characteristic of *S. pneumoniae*, confirmed by automated identification and susceptibility testing. The organisms from unit and recipient were serotype four.
The implicated RC unit had been collected ten days before transfusion. Venesection and processing had been uncomplicated. The donor was a 53 year old man, with no history of splenectomy or respiratory tract infection, who had not been vaccinated against *S. pneumoniae*. He had donated on numerous occasions prior to the index donation without complication. There had been no infective symptoms near the time of donation. Cultures of blood and of swabs obtained from nose, throat and both antecubital fossae were negative, as was urinary testing for pneumococcal antigen.
The ability of *S. pneumoniae* to survive in the refrigerated conditions of RC storage was explored. An expired RC unit was inoculated with 1×10^3 organisms of *S. pneumoniae* derived from the original cultures. The RC unit was maintained at 4ºC for ten days, following which samples were cultured and again demonstrated abundant growth of *S. pneumoniae*.
Conclusions: This case demonstrates that even organisms which are neither commensal nor psychrophilic may cause TTBI. The source of contamination could not be established definitively, but may have originated from transient donor bacteraemia. Even in retrospect no symptoms which might have led to donor deferral were identifiable. Prompt recognition of the source of sepsis enabled institution of appropriate therapy and liaison with the blood service to enable immediate recall of associated components.

P1708 Pneumococcal colonisation in the elderly in a non-outbreak setting

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Objectives: To study the prevalence, dynamics, and risk factors of pneumococcal nasopharyngeal colonisation in elderly subjects (n = 503, mean age = 80.3 ±10 SD) in the community (n = 109, mean age = 66.2±4.5 SD), nursing homes (n = 296, mean age = 84.3±7.4 SD), and the hospital (n = 98, mean age = 83.8±6.4 SD).
Methods: A nasopharyngeal swab (NPS) was taken through each nostril. The first NPS was directly plated on a selective blood agar and the second NPS after enrichment in broth. Pneumococci were identified using classical bacteriological techniques. A subset of nursing home residents colonised with pneumococci (n = 14) and negative controls (n = 26) were re-swabbed at 1, 2, 4, 8, and 12 weeks. In a subset of nursing home residents (n = 203, mean age: 84.4±7.1 SD) a PCR with a lytA gene probe was performed on DNA extracted from the primary NPS.
Results: The overall pneumococcal colonisation rate was 4.9% (25/503) (5.5% (6/109) in the community, 5.4% (16/296) in nursing homes and 3.1% (3/98) in hospital, P = NS). There were no significant differences in age and gender distribution, presence of comorbidities, vaccination status, hospitalisation and antibiotic use history, and functionality between colonised and non-colonized subjects. The broth enrichment technique on the second NPS yielded 28% (7/25) of the colonising pneumococci. Sixty-four % of the subjects initially colonised, carried a pneumococcus during the 3 month follow-up compared to 23% of the initially negative controls (P = 0.017). Compared to the PCR the bacterial culture technique had a sensitivity, specificity, positive predictive, and negative predictive value of 11.1%, 97%, 44.4%, and 83.5%, respectively.
Conclusions: Pneumococcal carriage-rate in the elderly, detected by bacteriological culture techniques, is low. Nursing home residents frequently carry pneumococci during a follow-up period of 3 months. In elderly subjects, the risk factors associated with pneumococcal carriage, the optimal bacteriological technique, and the value of molecular detection techniques need further study.

P1709 Serotype distribution and analysis of invasive *Streptococcus pneumoniae* isolates pre-7-valent pneumococcal conjugate vaccine introduction in the Republic of Ireland

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Objectives: The aims of this study were (1) to obtain data on serotype distribution of invasive *Streptococcus pneumoniae* (Spn) isolates, pre-7-valent pneumococcal conjugate vaccine (PCV7) introduction in the Republic of Ireland (RoI), (2) to determine the coverage provided by PCV7, and the 23-valent pneumococcal polysaccharide vaccine (PPV23) (3) to assess the proportion of invasive isolates with decreased susceptibility to penicillin (PNSP), and perform genotypic analysis of isolates.
Methods: Serotyping was performed using serological methods and multiplex PCR. Penicillin susceptibility was assessed by the E-test method (AB Biodisk, Solna, Sweden), following CLSI guidelines. MLST analysis was performed using standard procedures.
Results: Between April-November 2007, 161 invasive isolates were received from 25 laboratories nationwide. Thirty-one capsular serotypes were identified. The most common serotypes were 14 (n = 18), 7F (n = 14), 4 (n = 13), 6B (n = 11), 9V (n = 11), 19A (n = 11), and 1 (n = 10). In this study, 90% and 73% of isolates obtained from very young children (<2y) and children (<5y), respectively, were covered by PCV7; within the older population (⩾65y) 51% of isolates. An observed coverage of 88% occurred within the main target population (adults ⩾65y) for PPV23.
The incidence of PNSP was 12.4%; the most prevalent PNSP serotypes were: 9V (n = 8), 19F (n = 4) and 14 (n = 4). Preliminary MLST analysis of these isolates demonstrated the presence of ST156, ST2511 and ST1697, amongst others.
Conclusion: Based on epidemiological data to date, the introduction of PCV7 in the RoI is highly recommended. Continued surveillance of serotype distribution and the monitoring of PNSP isolates is of utmost importance, as the incidence of PNSP in the RoI is relatively high.

P1710 Epidemiological survey of invasive pneumococci in Hungary

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Objectives: *Streptococcus pneumoniae* is a major pathogen able to cause severe infections such as pneumonia, sepsis, or meningitis with high mortality rates if not treated correctly. The highest burden of disease is present in young children, and to protect them, the 7-valent conjugate vaccine (PCV7) was developed. As this vaccine was introduced in Hungary at the end of 2005, it was important to get an insight to the situation of pneumococci causing invasive infections in our country.
Methods: One hundred and six non-repeated invasive *Streptococcus pneumoniae* isolates were collected at seven different diagnostic laboratories throughout Hungary. The species identity of all strains was confirmed by the presence of the lytA gene. Their antibiotic sensitivity to 7 drugs was determined by agar dilution, according to the BSAC guidelines. Serotyping was done both conventionally with antisera and with a PCR-based method. The genetic relatedness of the strains was examined using pulsed-field gel electrophoresis (PFGE).
Results: We had 8 penicillin-resistant (R) strains among the isolates (8.4%, MIC = 2 or 4 mg/L), while there was virtually no resistance to cefotaxim or imipenem. The erythromycin resistance was 41.9%. The resistance to levofloxacin and moxifloxacin were around 3%. The isolates were fully sensitive to telithromycin and vancomycin. The detected serotypes in ranking order were: 6 (mostly 6A, 26.3%), 14 (17.1%), 23 (10.5%), 3 (9.2%), followed by 15, 9, 19, 7, 4, 18, 1, 11 and 8.

The penicillin-R strains were of serotypes 14, 23 or 19A. The serotype 23 strains required higher penicillin MICs, but were all sensitive to macrolides. The higher fluoroquinolone MICs were observed mainly for serotypes 3, 4, 18 and 19. Interestingly, serotypes 6, 9 and 23 were mostly isolated from pneumonia. Based on the PFGE results, the strains showed a relatively high genetic diversity.

Conclusions: The antibiotic sensitivity pattern of the strains was similar, but the serotype distribution was different from that previously observed in the general pneumococcal population in Hungary. Based on our data, the theoretical vaccine coverage for the PCV7 (serotypes 4, 6B, 9V, 14, 18C, 19F, 23F) is 51.3%, which is very low. This would increase to 59.2% with the soon coming 10-valent vaccine, and to 89.5% with the future 13-valent vaccine, thus this latter would be very welcomed in Hungary.

P1711 Phenotypic and genotypic characterisation of *Streptococcus pneumoniae* carriage strains from paediatric population in Arkhangelsk region, Russia

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Objectives: To determine the prevalence of the nasopharyngeal carriage of *Streptococcus pneumoniae* in children from Arkhangelsk region, Russia, and characterize the strains with regard to population structure and antimicrobial resistance.

Methods: A single nasopharyngeal (NP) swab from 438 non-vaccinated healthy children aged 1–7 years, at 10 day-care centres in the Arkhangelsk region in November 2006. *S. pneumoniae* isolates were identified by standard methods and examined for the susceptibility to oxacillin (OXA), tetracycline (TET), erythromycin (ERY), norfloxacin (NOR), and trimethoprim-sulfamethoxazole (TMP/SMX), with agar disk diffusion (Oxoid LTD, UK) using breakpoints defined by the Norwegian Working Group for Antibiotics (NWGA) and the Swedish Reference Group for Antibiotics (SRGA). OXA-resistant isolates were further examined for their susceptibility to penicillin G (PENG), cefuroxime, cefotaxime and meropenem by Etest. ERY-resistant isolates were examined by the double disk diffusion method with ERY and clindamycin (CLI) (Oxoid) as well as for erm(B) and mef-gen by PCRs. NOR-resistant isolates were examined by Etest for their susceptibility to ciprofloxacin (CIP), norfloxacin (NOR), moxifloxacin (MXE) and levofloxacin (LVX), using SRGA and EUCAST-breakpoints. Multi-drug resistance (MDR) was defined as resistance to ⩾3 groups of antibiotics. Ongoing analyses of the population structure are performed by serotyping and MLST.

Results: *S. pneumoniae* was recovered from 171 (39%) NP-samples. The rates of non-susceptibility were: TMP/SMX (n = 143;84%), TET (n = 54;32%), OXA (n = 31;18%), ERY (n = 18;11%), and NOR (n = 1; <1%). 22/31 (71%) of OXA-resistant strains were conformed non-susceptible for PENG (range 0.094–1 mg/L). Double disk diffusion analysis of ERY-resistant strains (n = 16) revealed the following phenotypes: iMLSB(n = 9), cMLSB (n = 4), and M-type (n = 3), which was confirmed by erm(B) and mef(A)-PCRs. One NOR-resistant strain revealed the following MIC profile: NOR⩾32 mg/L, CIP⩾4 mg/L, and susceptible to LVX (2 mg/L), MXE (0.25 mg/L). MDR was detected in 19 strains (11%).

Conclusions: (i)The prevalence of pneumococcal colonisation (39%) in children aged 1–7 years in Arkhangelsk is relatively high. (ii)High to moderate rates of non-susceptibility to commonly used antibiotics were observed: TMP/SMX (84%), TET (32%), PENG (13%) and ERY (11%) were observed. (iii)A low prevalence (<1%) of resistance to fluoroquinolones was observed.

P1712 Comparison of pneumococci isolated from invasive and non-invasive disease in pre-school children

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Objective: Characterisation of pneumococcal isolates recovered from invasive and non-invasive disease in children with less than 6 years, in Portugal.

Methods: *Streptococcus pneumoniae* is recognised as an important cause of morbidity and mortality especially among pre-school children. The infections caused by *S. pneumoniae* range in severity from mild infections such as otitis media to life threatening such as meningitis. Epidemiological studies have shown an association between certain serotypes and invasive infections. Nevertheless, serotype distribution varies considerably in terms of geographic localisation. In this work, we analysed and compared 72 isolates responsible for invasive and non-invasive disease in children with less than 6 years, recovered in 2003. Each isolate, was characterised using a combination of antimicrobial susceptibility profile, serotyping, macrorestriction profiling, using SmaI and pulsed-field gel electrophoresis (PFGE). Multilocus sequence typing (MLST) was performed for representatives of the main PFGE clones obtained.

Results: A significant modification of the distribution of serotypes in 2003 was observed, which included the absence of isolates expressing the main serotype found between 1999 and 2002, namely serotype 14. Nevertheless, molecular analysis did not show a radical change in clonal composition. Serotype distribution among non-invasive isolates differed significantly from the one found in the invasive population. Regarding molecular typing, no marked differences were found among the clonal composition. In terms of antimicrobial resistance, 88.5% and 40.0% of the invasive and non-invasive strains, respectively, were resistant to at least one of the antimicrobials analysed. Among the invasive collection, penicillin and erythromycin resistance decreased in 2003, which was associated with a decline of a small number of serotypes including some present in the heptavalent conjugate vaccine. Regarding non-invasive disease, while penicillin resistance also declined, erythromycin resistance increased considerably in 2003.

Conclusion: The results obtained in the present study indicate that the invasive pneumococcal population is changing in the age groups considered. The absence of serotype 14 isolates and associated clones was the most striking feature of this change, which was not observed among non-invasive isolates. The results indicate that, although related, the two populations are evolving along different lines.

P1713 emm-types distribution and susceptibility pattern of group A streptococci isolated from pharyngitis patients in Serbia

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Objective: Pharyngitis is one of the most frequent diseases caused by group A streptococci (GAS). Distribution of GAS emm types causing pharyngitis vary with time and geographical region. Although GAS remains susceptible to penicillin, an increased resistance to macrolides and other antibiotics has been noticed recently. The aim of this study was to investigate emm type distribution and susceptibility pattern of GAS isolates from patients with pharyngitis in Serbia.

Methods: Seventy pharyngeal isolates of GAS collected from various regions throughout Serbia were included in the study. The presence of emm genes was determined by PCR using "all M" primers following a previously published protocol by Podbielski and co-workers. Susceptibility to penicillin, erythromycin, clarythromycin, clindamycin, levofloxacin and tetracycline was determined by broth microdilution test according to the recommendations of CLSI standard, or on Phoenix automated system (BD, USA), using SMIC-10 panel.

Results: A total of 14 different emm types were identified among the tested isolates. The most common ones were emm 12 (21%), emm 6 (20%), emm 1 (18%), emm 3 (15%) and emm 4 and emm 28 (6% each). Resistance to erythromycin/clarythromycin was encountered in only 3%

of isolated strains. These isolates were also resistant to clindamycin indicating constutive MLS resistance, as well as to tetracycline. Overall, tetracycline resistance was recorded in 12% of isolates, while all strains were susceptible to levofloxacin.

Conclusion: The emm type distribution of pharyngeal isolates of GAS is similar to the reports from other parts of Europe, with the exception of relatively high prevalence of emm 6. Our results indicate that erythromycine resistance is not widespread in Serbia, despite its high prevalence in the neighbouring countries.

P1714 Comparison of macrolide-resistant and macrolide-susceptible populations of group A streptococci from asymptomatic oropharyngeal colonisation in Portugal

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Objectives: To assess the structure and evolution of macrolide resistant and susceptible populations of group A streptococci (GAS) from asymptomatic oropharyngeal colonisation during 2000 to 2005.

Methods: A total of 850 GAS collected from healthy carriers at day care centres (DCC) were tested for macrolide resistance frequency and phenotypes (M or MLSB) by disk diffusion. Out of these, all (n = 141) macrolide resistant (MR) isolates and part of the macrolide susceptible (MS) isolates [368 isolates collected at DCC where high (⩾15%) colonisation rates were observed] were characterised by SmaI or Cfr9I DNA-band pulsed-field gel electrophoresis (PFGE) profiling. Representatives of different PFGE patterns were characterised by T-typing, emm-typing and multilocus sequence typing (MLST).

Results: Resistance to macrolides was approximately 10% in 2000–02, 28% in 2003, 20% in 2004 and 3% in 2005. M phenotype isolates increased from 40% in 2000 to >80% during 2001–04 and were not detected in 2005. In parallel the MLSB phenotype isolates were prevalent in 2000 (60%) and in 2005 (100%).

Three major clonal lineages accounted for the majority of the M phenotype isolates (70% of 117 isolates) and were prevalent in different years: ST28 (emm1;emm107/T1/PFGE.CX) in 2001, ST36 (emm12/T12;others/PFGE.AP) in 2002/03 and ST39 (emm4;emmstMrp6/T4;others/PFGE.CZ) in 2004.

Also three major clonal lineages accounted for the majority of the MLSB phenotype isolates (63% of 24 isolates) and were as well detected in different years: ST46 (emm22;emm73/T13/PFGE.A) in 2000/01 and 2003, ST52/bacitracin-resistant (emm28/T28/PFGE.F) in 2003/04, and ST403 (emm11/T11;NT/PFGE.BO) in 2004/05.

Most of the MS isolates (69%) were included in six clones also variable in frequency and year of detection: ST55 (emm79/T2.12/PFGE.BH), ST382 (emm1;emm6/T6/PFGE.AD), ST36 (emm12/T3;other/PFGE.AP), ST403 (emm1/T1/PFGE.BG), ST28 (emm1/T1/PFGE.X), and ST-not assigned (emm12/T12/PFGE.AB).

Conclusions: The epidemiology of MR and MS GAS from asymptomatic colonisation changed rapidly during 2000–05. However, several lineages of MR and MS were identified which were described as prevalent and capable of causing a broad range of streptococcal infections in Europe. Two lineages (ST36 and ST403) were found to comprise MR and MS isolates of different T, emm and PFGE types. This study stresses the role of carriers as reservoirs of particular strains either resistant or susceptible to antimicrobials with high ability to colonize and disseminate.

P1715 Prevalence of group A streptococcal carriers in healthy school children

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Objectives: The aim of this study was to investigate the prevalence of nasopharyngeal *Streptococcus pyogenes* in asymptomatic school children in Afyonkarahisar, Turkey. A secondary aim was to investigate whether pharyngitis occurred among the asymptomatic carriers.

Methods: The study groups included 1129 primary school children in Afyonkarahisar, Turkey. An infectious disease specialist rubbed sterile swabs over the posterior nasopharyngeal walls of the 1129 children who had no symptoms of pharyngitis. Throat swabs were cultured for bacteria which were identified using standard microbiological methods. During the one year period of control, asymptomatic carriers were monitored for developing pharyngitis.

Results: Of the 1129 children 73 (6.5%) were isolated *S. pyogenes*. We diagnosed 4 (5.5%) cases of pharyngitis among group A streptococcal carries.

Conclusion: Our data shows that *S. pyogenes* nasopharyngeal carriage was found 6.5% in healthy school children. We observed a low occurrence of pharyngitis among asymptomatic school children.

P1716 Carriage of group B streptococcus in pregnant women from Tehran, Iran

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Objectives: Group B streptococcus (GBS) is the most common cause of life-threatening infection in neonates but is preventable if the mother is diagnosed before and treated at delivery. The objective of this study was to determine the colonisation rate of GBS in Iranian pregnant women.

Methods: Vaginal and anal swabs were obtained from 125 pregnant women at 35–37 weeks' gestation. Two anal and vaginal swabs were taken from each patient. One swab was inoculated to Todd-Hewitt broth supplemented with antibiotic. The other swab was used for a PCR assay, which uses specific primers for cfb gene sequence of GBS DNA.

Results: Out of 125 pregnant women, 12(9.6%) were GBS positive by PCR assay while culture identified 10(8%) women as carriage of Group B streptococcus. Using culture as the gold standard, the sensitivity of PCR assay was 100% and specificity was 98%. The positive predictive value (PPV) and negative predictive value (NPV) for PCR assay were 98% and 100%, respectively.

Conclusion: Group B streptococcus colonisation rate among Iranian pregnant women is relatively high (9.6%). The PCR method seems rapid, specific and highly sensitive assay to detect GBS in anal and vaginal samples taken from pregnant women. The results of this study, recommend screening of pregnant women at 35–37 weeks' gestation for GBS to reduce neonatal morbidity and mortality caused by this bacterium.

P1717 An outbreak of *Streptococcus agalactiae* catheter-related bacteraemia in a haemodialysis unit

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Introduction: Rates of invasive (Group B streptococcus, *Streptococcus agalactiae*) GBS disease in adults are on the rise. Factors associated with increased risk for community-acquired GBS infection, besides age, include cirrhosis, diabetes mellitus, stroke, breast or nonhaematologic cancer, decubitus ulcer, and neurogenic bladder. Nosocomial infection has been associated with age, placement of a central venous catheter (CVC), diabetes mellitus, congestive heart failure, and seizure disorder. We report an outbreak of GBS catheter-related bacteraemia in a hemodialysis (HD) unit.

Materials and Methods: Two patients with end-stage renal disease on hemodialysis done at the same outpatient hemodialysis unit were admitted on the same day in our hospital with catheter-related GBS bacteraemia. A retrospective study was undertaken at the HD unit to address risk factors for febrile illness on the last HD session day. A detailed questionnaire addressing risk factors for invasive GBS disease was completed by all HD patients treated on the same day with the 2 GBS patients (total of 12 HD patients) and by all members of the nursing and medical staff. Medical and nursing records of the HD unit

were reviewed as well as infection control and catheter care practices. All patients and staff members submitted rectal swabs for culture (females submitted vaginal swabs in addition).

Results: No rectal or vaginal culture of any HD patient or staff member was positive for GBS. One of the 2 GBS patients had a febrile syndrome that started 3 days before admission with documented high fever during the last 2 HD sessions while the other one developed fever a few hours after the last HD session. The two GBS patients received catheter care by 3 different nurses during the last 3 HD sessions before admission and their beds were not in immediate proximity. The probability of febrile illness on the day of last HD was associated with patient care for more than 30 minutes during at least 2 out of 3 most recent HD sessions by a specific nurse (p 0.045), while association with presence of CVC for HD did not reach statistical significance (p 0.09).

Conclusions: We speculate that the GBS strain (same antibiogram, PFGE not done) was transmitted from one patient to the other through the hands of medical personnel. No such outbreak has been ever reported in HD patients. The importance of strict infection control practices in HD units and avoidance of CVCs for long term HD should be emphasised.

P1718 Molecular identification of clinical isolates of *Nocardia* and related Actinomycetes in the French Observatory for Nocardiosis (OFN) – New epidemiology data of nocardiosis in France between 2000 and 2007

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Nocardiosis is a severe infection that usually affects immunosuppressed patients but also immunocompetent hosts. The number of patients suffering this infection is in regular increase in the world. *Nocardia* are aerobic Gram-positive actinobacteria which are in constant taxonomic evolution because of the development of new molecular tools that have lead the discovery of numerous new species; actually, this genus encompasses 69 species with validly published names. The French Observatory for Nocardiosis (OFN) is the expert centre in charge of the survey of nocardiosis since 1999. During these last years, the OFN is interested in developing specific molecular techniques to improve and to assure the best identification of pathogenic aerobic Actinomycetes.

We show the results of seven years of work in the nocardiosis surveillance. 607 strains were obtained from clinical isolates during the period 2000–2007. Species identification was performed by using partial sequencing of the 16S rDNA gene and some of these identifications were confirmed by the sequencing of hsp65 gene. 75% of the isolates belonged to *N. farcinica* (26%), *N. nova* (20%), *N. abscessus* (18%) and *N. cyriacigeorgica* (12%). The last 25% was formed by less incident species like *N. brasiliensis* (3%), *N. otitidiscaviarum* (3%), *N. transvalensis* (3%), *N. beijingensis* (2%), *N. veterana* (2%), *Nocardia* sp. strains not identified (2%) and other species (9%). In this last 9% OFN has identified up to 23 different species from where *N. asteroides*, *N. cerradoensis*, *N. paucivorans*, *N. shimofusensis*–*N. higofusensis*, *N. carnea*, *N. puris* and *N. pneumoniae* are the most common.

The average age of the patients was 63 years-old and sex-ratio was 1.6. The most common clinical specimens were collected from bronchoalveolar lavage (28%), pus (23%), sputum (19%) biopsy (7%), cerebrospinal fluid (3%) and other specimens (20%). The identification at genus level of other aerobic Actinomycetes was realised by using specific-PCR. Among these 273 strains collected, OFN identified different genus such as *Streptomyces* (54%), *Mycobacterium* (30%), *Gordonia* (6%) and other Actinomycetes (10%).

It clearly appears that the change of molecular tools modified dramatically the epidemiological data.

P1719 In vitro-activity of tigecycline and daptomycin against vancomycin-resistant enterococci and meticillin-resistant staphylococci

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Objective: Tigecycline, a glycylcycline and daptomycin, a lipopeptide are novel antibacterial compounds undergoing clinical development. The aim of this study was to assess the in vitro-activity of Tigecycline and Daptomycin against VRE and Meticillin-resistant Staphylococci from patients with positive cultures in our hospital.

Methods: A total of 32 clinical isolates of VRE (28 *E. faecium* and 4 *E. faecalis*) and 30 Staphylococci (20 MRSA and 10 MRSE) were isolated from various clinical specimens in one year. Identification was determined with the use of VITEK-II system (BIOMERIEUX-France). The antimicrobial susceptibility was determined by Kirby-Bauer, CLSI guidelines. Phenotypically all isolates of Enterococci were VanA type (MIC Vancomycin >256 μg/ml,MIC Teicoplanin >16 μg/ml). They were also susceptible to linezolid, except one, and expressed various susceptibility patterns to other antibiotics. All isolates of Staphylococci were susceptible to Vancomycin, Teicoplanin Linezolid and expressed various susceptibility patterns to other antibiotics. MICs were determined by E-test method (AB Biodisk). The susceptible breakpoints, according to CLSI guidelines were:.MIC Tigecycline ≤0.25 mg/l, MIC Daptomycin ≤4 mg/l for enterococci and MIC Tigecycline ≤0.5 mg/l, MIC Daptomycin <1 mg/l for Staphylococci.

Results: The isolates of enterococci and staphylococci were all susceptible to tigecycline. All the isolates of enterococci were susceptible to daptomycin, except one. All the MRSA and all the MRSE, except one, were susceptible to daptomycin.

Tigecycline MIC values:

For VRE faecium (28): 42.9% at 0.064 mg/l and 57.1% at 0.094 mg/l

For VRE faecalis (4): 75% at 0.064%mg/l and 25% at 0.094% mg/l

For MRSA(20): 20% at 0.19 mg/l, 40% at 0.25 mg/l, 35% at 0.38 mg/l and 5% at 0.5 mg/l

For MRSE (10): 20% at 0.064 mg/l, 20% at 0.094 mg/l, 10% at 0.19 mg/l, 20% at 0.25 mg/l, 10% at 0.38 mg/l and 20% at 0.5 mg/l.

Daptomycin MIC values:

For VRE faecium (28): 39.4% at 2 mg/l, 35.7% at 3 mg/l, 21.5% at 4 mg/l and 3.4% at 8 mg/l.

For VRE faecalis (40): 25% at 1 mg/l, 25% at1.5 mg/l, 25% at 2 mg/l and 25% at3 mg/l.

For MRSA (20): 5% at 0.38 mg/l, 10% at 0.5 mg/l, 45% at 0.75 mg/l and 40% at1 mg/l.

For MRSE (10): 11.2% at 0.38 mg/l, 22.2% at 0.75 mg/l and 66.6% at 1 mg/l.

Conclusion: These results suggest that both tigecycline and daptomycin may play important roles in the treatment of infections caused by VRE and meticillin-resistant staphylococci including drug-resistant strains.

P1720 In vitro activity of daptomycin against various VanA VRE species derived from clinical specimens

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Objectives: To evaluate the in vitro activity of daptomycin against a collection of Vancomycin Resistant *Enterococcus* (VRE) type VanA isolates belonging to various species, derived from clinical specimens.

Methods: We examined 48 VRE clinical isolates originated from diverse specimens (pus [n = 24], blood [n = 19], urine [n = 3] and bronchoalveolar excretions [n = 2]) of 48 patients treated in different wards of our hospital during a three year time period (2005–2007). These isolates were identified to species level using the automated system VITEK 2 (Biomerieux, France).

The enterococci were characterised as VanA phenotypically, by susceptibility to teicoplanin (i.e teicoplanin MIC of ≥32 mg/L, as probable VanA), and were confirmed genotypically by a sandwich hybridisation

method using the commercial EVIGENETM VRE detection kit (Statens Serum Institut, Denmark). The MIC levels of teicoplanin, vancomycin and daptomycin were determined using the E-test strips (AB Biodisk, Solna, Sweden) according to the manufacturer's recommendations. As sensitive to daptomycin VRE isolates were considered those with MIC < 4 mg/Ln in accordance to CLSI guidelines (issue M100 S17).
Results: The identification of the isolates showed *Enterococcus faecium* (n = 32), *Enterococcus faecalis* (n = 13), *Enterococcus avium* (n = 2) and *Enterococcus durans* (n = 1). All the examined VRE isolates belonged to VanA genotype. Both vancomycin and teicoplanin MIC values ranged between 96 and ⩾256 mg/L. The MIC levels of daptomycin varied between 1 mg/L and 4 mg/L. All the 4 VanA VRE species corresponding to the examined isolates were sensitive to daptomycin.
Conclusion: Daptomycin showed very good in vitro activity against this collection of various VanA VRE species. There were no resistant isolates to daptomycin regardless to enterococcal species. This new antibiotic could be an new option in the treatment of some infections caused by diverse VanA glycopeptide-resistant enterococcus species.

P1721 Evaluation of the in vitro susceptibility of meticillin-reistant staphylococcus strains isolated from nosocomial infection to vancomycin, teicoplanin, linezolid and tigecycline by E-test method

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Objectives: The aim of this study was to determine the MIC value of vancomycin, teicoplanin, tigecycline and linezolid susceptibilities of 100 meticillin-resistant staphyloccocus strains (21 meticillin-resistant *Staphylococcus aureus* (MRSA) and 79 meticillin-resistant coagulase negative staphylococcus (MR-CNS) isolated from nosocomial infection at Ankara Education and Researc Hospital in June 2005–March 2007.
Methods: The MIC values of 100 meticillin-resistant staphyloccus strain [(21 (MRSA), 79 (MR-CNS)] for vancomycin teicoplanin, linezolid and tigecycline were tested by E-test method. MIC values were determined according to the catalog of manufacturer (AB Biodisk, Sweden), Antibiotic susceptibilities were determined according to the recommendations of CLSI. For linezolid; strains MIC value of ⩽4 μg/ml were considered susceptible. For tigecycline strains with MIC value of ⩽0.5 μg/ml were considered susceptible.
Results: For overall 100 meticillin-resistant staphylococcus strains MIC values were as follows: for vancomycin MIC value 0.125–2 μg/ml range, mean 0.83 μg/ml, for teicoplanin MIC value 0.125–6 μg /ml range, mean 1.95 μg/ml, for linezolid MIC value 0.047–0.38 μg/ml range, mean 0.21 μg/ml, for tigecycline MIC value 0.064–0.38 μg/ml range, mean 0.28 μg/ml.
Conclusion: No resistance to vancomycin, teicoplanin, tigecycline and linezolid was established in meticillin-resistant staphylococcus strains isolated from the inpatients in our hospital and who had nosocomial infections. The most active antibiotic was established to be linezolid (mean MIC value 0.21 μg/ml) to be followed by tigecycline (mean MIC value: 0.28), vancomycin (mean MIC value:0.83) and teicoplanin (mean MIC value: 1.95), respectively. When glicopeptide antibiotics were compared with each other, MIC value of vancomycin was found to be lower than that of teicoplanin.
It is favourable that no resistance was found against glicopeptide group alternative antibiotics which are new treatment options in meticillin-resistant staphyloccus infections.
However, it should be borne in mind that infection control measures, surveillance of the antibiotic susceptibility results and judicious use of antibiotics are more important than the advent of new antibiotics in the prevention of the development of resistance to antibiotics and of nosocomial infections.

P1722 Efficacy of ertapenem against staphylococci isolates

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Ertapenem is a new carbapenem with excellent activity against different Gram-negative and Gram-positive bacteria and a long serum half-life. Its good antibacterial activity is closely related to high hydrolysis resistance by bacterial β-lactamases. Acquired resistance to ertapenem is usually mediated by up-regulation of efflux mechanisms and by the selection of porin-deficient mutants.
Objectives: The aim of this study was to evaluate antimicrobial activity of ertapenem by comparison that of meticillin, gatifloxacin, cefepime and linezolid against 455 staphylococci collected during 2006–2007 period from healthy individuals.
Material and Methods: Microorganisms: A total of 455 staphylococci, 214 coagulase-positive staphylococci (CPS) and 241 coagulase-negative staphylococci (CNS), colonising skin and nasal mucous, were obtained from medical personal during 2006–2007. Isolates were identified as CPS by demonstrating a positive coagulase test.
Antimicrobial agents: Powders of ertapenem, meticillin, gatifloxacin, cefepime and linezolid obtained from commercial sources were tested in concentration range from 0.125 to 128 mg/l.
Sensitivity study: Minimum inhibitory concentrations (MICs) were determined by the agar dilution method established by NCCLS guidelines.
Results: Ertapenem was very active agent against staphylococci with MICs at which 99% of isolates are inhibited of ⩽2 mg/L (susceptibility breakpoint) for both coagulase-positive and coagulase-negative staphylococci. Resistance to ertapenem was observed in four of all tested staphylococci strains.
Conclusion: Ertapenem has an activity comparable to the other tested agents. Resistance rate was 1% for all investigated staphylococci strains.

Epidemiology of antimicrobial resistance among Gram-positives

P1723 German surveillance study on the in vitro activity of daptomycin against Gram-positive cocci

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Objectives: Drug resistance (R) in Gram+ bacteria has become an increasing problem over the past 20 years. New antimicrobial agents such as the lipopeptide daptomycin (DAP) have been developed to address this problem. Shortly after the approval of DAP in the EU, a surveillance study was started by a network of 73 laboratories to monitor the susceptibility of Gram+ bacteria to DAP in Germany. The objective of this study was to evaluate the susceptibility of isolates of six clinically important Gram+ species to DAP and other antimicrobial agents.
Methods: A total of 2,646 isolates incl. meticillin-susceptible *S. aureus* (MSSA, n = 661), meticillin-resistant *S. aureus* (MRSA, n = 439), *S. epidermidis* (Se, n = 425), *E. faecalis* (Es, n = 341), *E. faecium* (Em, n = 324), *S. pyogenes* (Spy, n = 319) and *S. agalactiae* (Sag, n = 137) were tested against DAP, linezolid (LZD), vancomycin (VAN) and other drugs. Primary isolates obtained from hospitalised patients with skin and soft tissue infections, respiratory tract infections, foreign body/catheter infections or sepsis were included. MICs were determined in a central laboratory using the microdilution method according to the German DIN standard. For DAP, the Ca2+ concentration of the test solution was adjusted to 50 mg/L. MICs were interpreted by EUCAST criteria, when possible.
Results: Isolates were primarily recovered from wound swabs (65%), blood samples (21%) and respiratory specimens (9%). MIC50/90s of DAP for MSSA, MRSA, and Se each were 0.5/1 mg/L. All but one isolates were inhibited by DAP at the breakpoint of 1 mg/L. The remaining isolate had an MIC of 2 mg/L. All staphylococci were susceptible to LZD and VAN. The highest MIC of VAN for MRSA was 2 mg/L. Based on MIC50/90s, DAP was up to 4-times more active than LZD or VAN against staphylococci.

Of the Em and Es isolates, 33 (10%) and one (0.3%) were R to VAN, respectively. DAP inhibited all strains at 4 mg/L. One Es isolate was resistant to LZD (MIC 32 mg/L). High-level R to gentamicin (MIC > 500 mg/L) was observed in 35/42% of Es/Em isolates.

DAP, with an MIC50/90 of 0.125/0.25 mg/L, was more active than LZD (1/1 mg/L) or VAN (0.25/0.5 mg/L) against Spy. Against Sag, DAP (0.5/1 mg/L) was more active than LZD (1/2 mg/L) and comparably active to VAN (0.5/0.5 mg/L). R to erythromycin was detected in 9% of Spy isolates and 20% of Sag isolates.

Conclusions: DAP demonstrated excellent in vitro activity against a large collection of German Gram+ cocci including MRSA and VRE.

P1724 Gram-positive anaerobic cocci are resistant to penicillin and clindamycin

C.E. Nord for the ESCMID Study Group on Antimicrobial Resistance in Anaerobic Bacteria

Objectives: Gram-positive anaerobic cocci (GPAC) are a heterogeneous group of microorganisms. GPAC are involved in one-quarter of all anaerobic isolates from human clinical infections. The prevalence of GPAC as pathogens is increasing and information regarding their antimicrobial susceptibilities is relatively limited compared with that of other anaerobic species. The aim of this study was to examine the current status of antimicrobial resistance in clinical isolates of GPAC in Europe.

Methods: Collaborators in each participating country (Austria, Croatia, Czech Republic, Denmark, Finland, France, Great Britain, Greece, Hungary and Sweden) collected consecutive clinical isolates of GPAC. The isolates were identified by Gram staining, biochemical tests and gas–liquid chromatography. Minimum inhibitory concentrations of penicillin, clindamycin, metronidazole, vancomycin and linezolid were determined by the agar dilution method according to the Clinical and Laboratory Standards Institute. Production of β-lactamase was determined by the nitrocefin test.

Results: 299 strains were identified as GPAC and 23 isolates as other species. Most isolates were collected in Sweden (22%), Finland (19%) and Great Britain (18%). The majority of isolates were identified as *Finegoldia magna* (111), *Parvimonas micra* (53), *Peptoniphilus harei* (44), *Anaerococcus vaginalis* (21) and *P. anaerobius* (20). Nineteen isolates were identified as *Peptostreptococcus* sp. The remaining isolates were identified as *Peptoniphilus asaccharolyticus* (8), *Peptoniphilus ivorii* (5), *Peptoniphilus indolicus* (8), *Peptoniphilus lacrimalis* (5), *Anaerococcus octavius* (1), *Anaerococcus prevotii* (2), *Anaerococcus tetradius* (1) and *Anaerococcus lactolyticus* (1). All isolates were susceptible to metronidazole, vancomycin and linezolid. A total of 21 isolates were resistant to penicillin and/or clindamycin. The majority of resistant strains were collected in Great Britain. Eight of the isolates were identified as *F. magna*, of which four were resistant to clindamycin, three to both penicillin and clindamycin and one isolate to penicillin. No isolates produced β-lactamases. The origins of resistant isolates were blood, abscesses and soft tissue infections.

Conclusion: Antimicrobial susceptibility testing of anaerobic cocci in patients with severe infections as well as continuous surveillance of antimicrobial susceptibility in GPAC seem highly justified.

P1725 Daptomycin activity and spectrum when tested against Gram-positive species responsible for bloodstream infections in European medical centres

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Objective: As part of the Daptomycin Surveillance Program, we evaluated the in vitro activity of daptomycin against clinical Gram-positive organisms isolated from patients with bloodstream infections (BSI) in Europe. Daptomycin is a novel cyclic lipopeptide approved by European Health Authorities for the treatment of complicated skin and soft tissue infections (cSSTI), right sided infective endocarditis (RIE) due to *S. aureus* and for *S. aureus* bacteraemia (SAB) when associated with RIE or with cSSTI.

Methods: BSI isolates (11,764) were collected from 32 medical centres located in Europe (13 countries) and Israel in 2003–2007. The following pathogens were evaluated: *S. aureus* (28.3% oxacillin [OXA]-resistant [R]); coagulase-negative staphylococci (CoNS; 77.4% OXA-R), *E. faecalis* (EF; 1% vancomycin [VAN]-R), *E. faecium* (EFM; 14.9% VAN-R), beta-haemolytic *Streptococcus* spp. (BHS; 627), and *viridans* group *Streptococcus* spp. (VGS; 497). The strains were susceptibility (S) tested by broth microdilution methods in cation-adjusted Mueller-Hinton broth, additionally supplemented to 50 mg/L of calcium for daptomycin tests. Numerous comparators were also tested.

Results: Daptomycin was highly active against *S. aureus* and CoNS (MIC90, 0.5 mg/L) and the activity was not adversely affected by co-resistance to OXA. Only 1 *S. aureus* strain was non-S to daptomycin (MIC, 2 mg/L). *E. faecalis* showed high rates of S to daptomycin (100.0%), linezolid (100.0%), ampicillin (99.7%) and VAN (98.9%), whereas *E. faecium* showed low S to VAN (82.9%). Daptomycin (100.0% S) was the most active compound tested against VAN-R *E. faecium*, followed by linezolid (98.4% S) and quinupristin/dalfopristin (73.3% S; most non-S strains were from France). Rates of VAN-R *E. faecium* increased from 10.7% in 2003 to 20.4% in 2007, and were highest in Ireland, Israel and the United Kingdom (36.1–37.5%). Elevated rates of high-level gentamicin (33.6%) and streptomycin (42.2%) R were observed among enterococci and daptomycin was the only compound active against all enterococcal strains at the S breakpoint. Daptomycin was also very active against BHS and VGS (MIC90, 0.25 and 0.5 mg/L respectively).

Organism (no. of isolates)	Cumulative % inhibited at daptomycin MIC (mg/L) of:						No. of non-S strains (%)[a]
	≤0.12	0.25	0.5	1	2	4	
S. aureus (5,324)							
OXA-S (3,816)	5.3	82.6	99.6	>99.9	100.0	–	1 (<0.1)
OXA-R (1,508)	2.5	64.2	98.7	100.0	–	–	0 (0.0)
CoNS (3,232)	8.4	61.2	96.9	99.8	>99.9	100.0	6 (0.2)
E. faecalis (1,345)	0.7	5.9	53.2	96.1	99.9	100.0	0 (0.0)
E. faecium (739)							
VAN-S (613)	0.8	1.3	6.2	30.8	78.5	100.0	0 (0.0)
VAN-R (126)	0.0	1.6	7.9	41.3	87.3	100.0	0 (0.0)
BHS (627)	81.8	97.9	100.0	–	–	–	0 (0.0)
VGS (497)	32.8	63.4	93.0	99.8	100.0	–	1 (0.2)

[a]US-FDA/CLSI interpretive criteria.

Conclusions: Daptomycin showed significant potency and broad-spectrum activity against recent clinical Gram-positive isolates collected from European medical centres, including MDR subsets. R to other compounds did not adversely influence daptomycin potency against staphylococci, enterococci or streptococci.

P1726 Quantifying factors associated with changes in antibiotic susceptibility patterns of enterococci isolated from dairy cattle

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Objective: The objective of this study was to quantify factors associated with changes in antibiotic susceptibility patterns in enterococci isolated from bovine faeces over a 2.5-year longitudinal study. We hypothesised that increasing levels of resistance would be positively associated with prior antibiotic treatment and younger animals. We also hypothesised that farms with the highest overall use of antibiotics would have the highest levels of resistance, but that the specific resistance patterns would vary among species of enterococci.

Methods: Four dairy farms were chosen in central Illinois, USA, and a cohort of approximately 50 animals of different ages was randomly selected on each farm. Faecal samples were collected from each animal every three months. Multiplex PCR was used to speciate the isolates into *E. faecium*, *E. faecalis*, *E. hirae* or *Enterococcus* spp. For each isolate, minimum inhibitory concentrations (MICs) for 17 antimicrobial agents

were determined using microbroth dilution and CLSI-recommended control strains.

Results: *E. faecium* (n = 1481) and *E. hirae* (n = 1355) were the predominant species isolated. Resistance was found more commonly in *E. faecium* isolates compared to *E. hirae* isolates for bacitracin (78%, 5%), ciprofloxacin (16%, 1%), erythromycin (14%, 1%), and quinupristin/dalfopristin (24%, 11%). Decreased susceptibility to flavomycin was observed in 88% of all *E. faecium* and 84% of *E. hirae* isolates. Tetracycline resistance was more common in *E. hirae* isolates than *E. faecium*, occurring in 54% and 31% of isolates respectively. Various resistances, including tetracycline and ciprofloxacin, were positively associated with the amount of antibiotic used on the farm. Calves were more likely to have resistance enterococci than older animals.

Conclusions: At the farm level, the prevalence and diversity of resistance was greatest in those farms using the most antibiotic. During the study, resistance levels did not change significantly within the farm. As seen in other studies, young animals had bacterial populations with more resistance, and this appeared to be independent of antibiotic treatment. Although *E. faecium* and *E. hirae* were exposed to the same selection pressures, there were noticeable differences in the resistance patterns between them. With many isolates having multidrug resistance, the choice of antibiotic on the farm becomes more challenging knowing that a single antibiotic can co-select for multiple resistances.

P1727 A comparison of antimicrobial susceptibility and MIC-distributions in *Enterococcus* isolates originating from humans, animals and retail meat

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Objectives: The National Antimicrobial Resistance Monitoring System (NARMS) is a collaborative effort between the U.S. Food and Drug Administration's Center for Veterinary Medicine (FDA CVM), U.S. Department of Agriculture (USDA), and the Centers for Disease Control and Prevention (CDC). The NARMS programme monitors resistance among zoonotic pathogenic (*Salmonella* and *Campylobacter*) and commensal (*Escherichia coli* and *Enterococcus*) bacteria recovered from food animal, retail meat and human sources. Enterococcal data from the NARMS programme has however, yet to be compared among these sources. In this study, we report on antimicrobial susceptibility and MIC-distributions of *Enterococcus* isolates recovered from humans, chickens at slaughter and retail meat.

Methods: The first 100 consecutive isolates of *Enterococcus faecium* and *Enterococcus faecalis* from each source submitted in 2005 were included. Minimum inhibitory concentrations (MIC) for 12 antimicrobial agents were determined using broth microdilution methods and interpreted according to CLSI standards, where available. MIC-histograms were constructed and compared with wild type distributions proposed by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (www.eucast.org). For each distribution, the MIC50 was calculated.

Results: There was good agreement among MIC-distributions of human, chicken and retail meat isolates. For the majority of antimicrobial agents, the MIC50 of the three distributions agreed or was within one dilution step. The most pronounced difference was observed for *E. faecium* and tetracycline. The MIC50 of human *E. faecium* isolates was ⩽4 mg/L whereas the MIC50 for both the chicken and retail meat isolates was 32 mg/L. MIC-distributions of susceptible isolates from all three U.S. sample sources correlated well with the wild type distributions proposed by EUCAST.

Conclusion: The MIC50 for all tested antimicrobial agents were similar for all enterococcal isolates, regardless of origin, with the exception of tetracycline where the MIC50 was higher for chicken and retail meat isolates. Additionally, MIC-distributions of susceptible enterococci from all three U.S. sample sources agreed well with the wild type distributions defined by EUCAST. This implies that the enterococcal wild type distributions are similar regardless of geographic origin and sample source.

P1728 Activity of tigecycline tested against vancomycin-resistant enterococci, including clonal complex-17 *E. faecium* strains, isolated in Europe

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Objectives: To evaluated the activity of tigecycline when tested against clinical strains of enterococci collected in European medical centres, including *E. faecium* strains characterised as being clonal complex 17 (CC-17).CC-17 characterizes a lineage of *E. faecium* with resistance (R) to ampicillin (AMP) and ciprofloxacin (CIP) with or without R to vancomycin (VANC), a pathogenicity island and an association with hospital outbreaks, which have spread globally.

Methods: Enterococcal strains were submitted from 34 medical centres located in Europe (13 countries) and Israel during 2000–2007. Strains were susceptibility (S) tested against tigecycline and >20 antimicrobials using CLSI broth microdilution methods and US-FDA/EUCAST interpretative criteria (tigecycline S at ⩽0.25 mg/L). VanA *E. faecium* strains R to AMP and CIP were characterised as a CC-17. A subset of CC-17 strains were further characterised by PFGE and PCR for esp gene.

Results: 4,591 enterococcal strains (7.1% VANC-R) were collected, including 3,070 *E. faecalis* (1.6% VANC-R) and 1,337 *E. faecium* (22.3% VANC-R). VANC-R was observed in 27 (79.4%) centres (all countries surveyed). Higher rates of VANC-R were found in Germany, Ireland and the United Kingdom. High level gentamicin R was observed in 33.3% of *E. faecalis* and 40.9% of *E. faecium*, while only 73.5% of *E. faecium* were S to quinupristin/dalfopristin. Tigecycline was very active against enterococci (MIC90, 0.25 mg/L; 97.3% S), including VANC-R *E. faecium* (MIC90, ⩽0.12 mg/L; 99.7% S) and *E. faecalis* (MIC90, 0.25 mg/L; 94.0% S) strains (see Table). Tigecycline was also highly active against VANC-R *E. faecium* CC-17 phenotype strains (MIC90, ⩽0.12 mg/L; 100.0% S).

Organism (no. tested)	Cumulative % inhibited at TIG[a] MIC (mg/L) of:				TIG non-S isolates
	⩽0.12	0.25	0.5	1	No. (%)
All Enterococci (4,591)	75.5	97.3	>99.9	100.0	124 (2.7)
E. faecalis					
VANC-S (3,020)	68.6	96.9	99.9	100.0	95 (3.1)
VANC-non-S (50)	60.0	94.0	100.0	–	3 (6.0)
E. faecium					
VANC-S (1,039)	89.9	98.6	99.8	100.0	15 (1.4)
VANC-non-S (298)	94.6	99.7	100.0	–	1 (0.3)
CC-17 VANC-R phenotype (162)	96.3	100.0	–	–	0 (0.0)

[a]TIG = tigecycline.

Conclusions: Tigecycline was very active against strains of enterococci causing infections in European hospitals and its activity was not adversely affected by R to VANC or other antimicrobials. This novel glycylcycline represents an important therapeutic option for infections caused by vancomycin-resistant enterococci, including the epidemic *E. faecium* CC-17 strains.

P1729 In vitro evaluation of tigecycline against drug-resistant Enterococci and *S. aureus* in the United States

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Background: Tigecycline (TIG), a member of a new class of antimicrobials (glycylcyclines), has been shown to have potent activity against many Gram-positive and -negative organisms. The T.E.S.T. Program determined the in vitro activity of TIG against *S. aureus* and enterococci resistant to 10 commonly prescribed antimicrobials:

amoxicillin-clavulanic acid (AUG), piperacillin-tazobactam (PT), levofloxacin (LVX), ceftriaxone (CAX), linezolid (LZD), minocycline (MIN), vancomycin (VAN), ampicillin (AMP), penicillin (P) and imipenem (IMP). Study strains were collected from hospitals globally throughout 2004–2006.

Methods: A total of 7,343 clinical isolates (2,903 enterococci, 4,440 *S. aureus*) from 193 labs in the United States were identified to the species level at each participating site and confirmed by the central laboratory. Minimum Inhibitory Concentrations (MICs) were determined by the local laboratory using broth microdilution panels. Antimicrobial resistance was interpreted according to CLSI and FDA (TIG) breakpoints.

Results: 840 (29%) enterococci and 1706 (38%) *S. aureus* (including MR + MS strains) were resistant to two or more drug classes. Among the enterococci, resistance rates were LVX 99%, P 85%, AMP 84%, VAN 76%, MIN 9%, and LZD 0%. Resistant rates for *S. aureus* were P 100%, AMP 100%, AUG 74%, LVX 99%, PT 63%, CAX 44%, IMP 15%, LZD 0%, MIN 0.2% and VAN 0%. TIG inhibited 100% of all the enterococci and *S. aureus* resistant to other drugs. Modal TIG MICs ranged between 0.06 and 0.12 μg/ml and were generally the same for most resistant and susceptible strains.

Conclusions: TIG retained potent activity against drug-resistant *S. aureus* and enterococcal isolates, inhibiting 100% of all resistant strains tested. TIG should prove to be a useful drug for therapy of infections with these resistant Gram-positive pathogens.

P1730 Cost of bacteraemia caused by meticillin-resistant *Staphylococcus aureus* in Spain

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Objectives: Meticillin-resistant *Staphylococcus aureus* (MRSA) is associated with complicated infections. Hospitalisation costs associated with MRSA infection are higher than those associated with meticillin-sensitive *Staphylococcus aureus* (MSSA). Limited information about costs from bacteraemia caused by MRSA is available. This study aimed to calculate the impact of MRSA bacteraemia on healthcare resources and associated costs in Spain.

Methods: Multicentre retrospective observational case–control study of the use of healthcare resources and the associated costs in the treatment of infections caused by MRSA was carried out. The results obtained on the use of resources enabled us to calculate the costs of a bacteremic episode caused by resistant or sensitive strains. Data on the use of resources were collected over a 12-month period (Jan-Dec 2005). Patients with MRSA and MSSA bacteraemia admitted in Spanish hospitals were included. Patients' clinical characteristics were recorded and comorbidity was assessed by Charlson index. The study was carried out from the perspective of the National Health Service; therefore, only use of healthcare resources was recorded. The costs of bacteraemia were expressed as direct healthcare costs. Qualitative and quantitative descriptive analyses were made for all the variables.

Results: We reviewed the clinical histories of 366 patients (28 hospitals) with bacteraemia *S. aureus* (121 MRSA and 245 MSSA). No differences in comorbidity were observed, although there was a greater presence of septic shock in the MRSA group. Prior antimicrobial therapy was administered in 47.1% and 7.8% of patients with and without MRSA, respectively ($p < 0.001$). The differences in the total length of antibiotic therapy were not statistically significant. Patients with MRSA had a longer hospital stay (8.2d more) and a higher ICU admission rate (7.6% more). The average cost per episode of bacteraemia rose to €14,854.89 (MRSA) and €11,079.54 (MSSA) difference of €3,775.35; 1.34 times greater.

Conclusions: Treatment of infections caused by MRSA generates an additional cost of €3,775.35 per episode of bacteraemia. The costs of an episode of MRSA bacteraemia increased 1.34 times compared with MSSA. This increase in costs related to MRSA bacteraemia is due mainly to the greater rate of admissions to the ICU and the longer length of stay.

P1731 Evolution of glycopeptide MICs in MRSA and MSSA isolates in a Spanish hospital between 2002 and 2006

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In recent years increased MICs of vancomycin has been described in meticillin-resistant *Staphylococcus aureus* (MRSA). The objetive of this study was to evaluate MIC trends of vancomycin and teicoplanin in clinical MRSA and meticillin-susceptible *S. aureus* (MSSA) isolates over a 5 year period (2002–2006).

MICs of vancomycin and teicoplanin were studied by broth microdilution in 567 MRSA and 2,575 MSSA clinical strains (one per patient) isolated between 2002 to 2006. BHI broth with 4 mg/L of vancomycin was used to screen vancomycin resistance. MICs of each antibiotic obtained in the different years was compared by the linear trend chi-square test.

All isolates were susceptible to vancomycin and teicoplanin. No statistically significant diference was observed in vancomycin for MRSA (X2 = 0.01; $p = 0.91$), and for MSSA (X2 = 0.08; $p = 0.78$). No statistically significant diference was observed in teicoplanin for MRSA (X2 = 3.06; $p = 0.08$), but a statistically significant trend to more susceptibility was observed in teicoplanin for MSSA (X2 = 5.19; $p = 0.02$).

Consumption of parenteral vancomycin in our Hospital in DDD/100 stays were: 2002 (1.91), 2003 (1.63), 2004 (1.74), 2005 (2.06), and 2006 (1.64). Oral vancomycin consumption for selective digestive decontamination in DDD/100 stays of ICU patients were: 2002 (63.5), 2003 (54.2), 2004 (49.5), 2005 (44.4), and 2006 (58.2).

In a scenary of few variations of vancomycin consumption, we did not notice important changes in vancomycin MICs in MRSA and MSSA clinical isolates over the period studied.

P1732 Decling susceptibility of *S. aureus* and coagulase-negative staphylococci to vancomycin during an 18-year period in Denmark

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Objectives: Several authors have reported treatment failure with vancomycin for staphylococci with MICs ⩾4 mg/l. and it has been proposed to lower the breakpoint for sensitivity to vancomycin for staphylococci from 4 mg/l to 2 mg/l. The aim of the present study was to elucidate the trend over time of the susceptibility of *S.aureus* and coagulase-negative staphylococci (CNS) to vancomycin at Herlev University Hospital.

Methods: Herlev University Hospital is receiving specimens from the primary and secondary sector from the former Copenhagen County. During the 18 year period 1990–2007 vancomycin susceptibility testing of *S. aureus* and CNS was performed either by a disc diffusion method using Oxoid discs or by MIC determination with E-test (Biodisc). The disc diffusion method was applied to 2173 isolates of *S.aureus* and 9857 isolates of CNS and MIC determination was done on 1245 isolates of *S. aureus* and 1989 isolates of CNS. Results from the three periods 1990–1995, 1996–2001 and 2003–2007 were compared.

Results: The mean vancomycin zone for *S. aureus* decreased from 20.6 to 16.0 mm ($p < 0.001$) from the first time period till the last. For CNS a decrease was observed from 21.8 mm to 16.8 mm ($p < 0.001$). In the first two periods all *S. aureus* isolates had MICs ⩽2 mg/l as compared to only 62.6% in the last period. In the last period 21.5% of the isolates had MICs ⩾4 mg/l. The proportion of MICs ⩽2 mg/l for CSN decreased from 77.8% in the first time period to 41.0% in the last period. In the last period 40.0% of CNS had MICs ⩾4 mg/l.

Conclusion: Both *S. aureus* and CNS showed a declining sensitivity to vancomycin during the 18 year period. A trend which is worrying considering the reported risk of treatment failure when the MIC to vancomycin is ⩾4 mg/l. This applied to 40% of CNS and 20% of

S.aureus investigated and may be a problem especially when treating infections in difficult accessible foci.

P1733 The prevalence of fluroquinolone resistance amongst staphylococci isolated from houshold pets

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Objectives: The prevalence of fluoroquinolone resistance using enrofloxacin as a marker was investigated amongst 21,261 isolates of staphylococci from animal origin. This was followed by the identification and characterisation of staphylococcal isolates over a 3-month period at IDEXX Laboratories. These isolates were then subjected to antimicrobial susceptibility testing against a range of 9 fluoroquinolones. These isolates were also examined for evidence of cross-resistance with other classes of antimicrobials.

Methods: Identification was initially carried out using recognised routine laboratory methods, plus the use of the VITEK automated system. This was then corroborated using ribotyping. The fluoroquinolone susceptibility testing was done using disc diffusion against the antimicrobials marbofloxacin, enrofloxacin, ciprofloxacin, pefloxacin, moxifloxacin, lomefloxacin, levofloxacin, ofloxacin and norfloxacin. Analysis of cross-resistance was conducted using sensitivity data from the VITEK analyser.

Results: Fluoroquinolone resistance was found to in the order of 6% among all staphylococcal isolates. It was most prevalent in meticillin-resistant *Staphylococcus aureus* (MRSA) isolates (93.4%), followed by coagulase-negative staphylococci (CNS) (10.9%), *S. aureus* (3.7%) and finally *S. intermedius* (2.7%). The identification methods used were found to correlate well with DNA ribotyping. The order of efficacy gained for the range of fluoroquinolones was ofloxacin (least active), through pefloxacin, norfloxacin, lomefloxacin, ciprofloxacin, enrofloxacin, marbofloxacin, levofloxacin to moxifloxacin (most active). Cross, or multi-resistance was taken as resistance to 3 or more classes of antimicrobials. It was seen most often in *S. intermedius*, followed by CNS and MRSA. Cross-resistance was not seen in isolates of MSSA or S. *schleiferi* ssp. *coagulans*.

Conclusion: The results gained from this project correlate well with those of previous studies. This indicates that staphylococci of animal origin appear to follow similar resistance patterns to human isolates. Further research into CNS isolates from animals is necessary due to the low number of isolates obtained.

P1734 Antibiotic susceptibility of *Staphylococcus epidermidis* isolated from prosthetic joint infections with special focus on rifampicin and variability of the rpoB gene

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Objectives: *Staphylococcus epidermidis* is a commensal that comprises a substantial part of the normal skin flora of humans. Nevertheless, this bacterium has emerged as the most important pathogen in infections related to implanted foreign body materials, especially prosthetic joint infections (PJIs).

A major problem with the treatment of PJIs caused by *S. epidermidis* is the ability of the bacterium, when accumulating on a surface such as an implant, to produce biofilm. The biofilm reduces the accessibility of antibiotics and host immune components such as complement and antibodies. Cell wall antibiotics such as isoxazolyl-penicillin or glycopeptides have not been successful agents for treatment of PJIs. On the contrary, rifampicin has the ability to penetrate the biofilm, is active also against stationary phase bacteria, and hence has emerged as an effective antimicrobial agent for PJIs. However, resistance to rifampicin may rapidly emerge and rifampicin monotherapy should be avoided. Accordingly, effective antibiotics for combination therapy with rifampicin are needed.

The aim of this study was to investigate the antimicrobial activity of various antibiotics against *S. epidermidis* isolated from PJIs, with special focus on rifampicin and the molecular mechanisms for development of rifampicin resistance.

Methods: Thirty-three *S. epidermidis* isolates obtained during revision surgery due to PJIs with extraction or exchange were analysed. The minimum inhibitory concentrations (MICs) of 16 different antibiotics were determined using the Etest method. A real-time PCR followed by sequencing of a 1052 bp segment of the rpoB gene was used.

Results: Among the rifampicin resistant isolates (39%), one or two single nucleotide polymorphisms (SNPs) in rpoB were identified, with one exception.

Furthermore, 79% of the isolates were resistant to ciprofloxacin, 79% to gentamicin, 67% to clindamycin and 39% to fucidic acid. All of the isolates were susceptible to linezolid and tigecycline, and 97% to daptomycin. The MIC50 and MIC90 of dalbavancin was 0.032 and 0.047 μg/ml and of ceftobiprole 0.5 and 1.5 μg/ml, respectively. mecA was detected in 85% of the isolates.

Conclusion: *S. epidermidis* isolates causing PJIs are often multiresistant including rifampicin. The rifampicin resistance is mainly caused by one or two SNPs. However, some of the newer antimicrobial agents may provide potential alternatives for monotherapy or combination therapy with rifampicin.

P1735 Trends of meticillin-resistant *Staphylococcus aureus* prevalence in ambulatory care in the Berlin area, 2000–2006

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Objectives: Surveillance data prove an increase in meticillin-resistant *Staphylococcus aureus* (MRSA) prevalence in German hospitals since the 1990s. Little is known about the situation in ambulatory care settings in Germany. A dataset from a laboratory serving for physicians in the city and surroundings of Berlin covering the period from 2000 to 2006 was analysed.

Methods: *Staphylococcus aureus* identification and antimicrobial susceptibility testing (AST) were performed by VITEK 1 system (bioMerieux). The phenotypical detection of MRSA was confirmed by subsequent PCR for MecA gene. Trends stratified for disciplines (internal medicine, general medicine, surgery, paediatrics, gynaecology and ear, nose and throat) and materials were calculated.

Results: In 12,723 (10.1%) out of 125,822 clinical specimens a *S. aureus* was detected. For 11,993 (94.3%) isolates AST was carried out resulting in an average MRSA proportion of 13.2 percent. From 2000 to 2006 the overall percentage of MRSA in ambulatory care increased from three percent to 17 percent with a yearly increment of 2.4% (CI: 2.0–2.8). With regard to specimen type the sample was composed as follows: 37.0% of the isolates came from wound swabs, 26.3% from swabs of unknown sites, 26.2% from nose and throat swabs, 7.0% from urines, and 3.5% from sputum. MRSA proportions ranged from 11.1% in urines to 14.8% in nose and throat swabs. Results can be stratified by the specialty of the physician who sent the specimen: MRSA proportions differ significantly with highest proportions in internal medicine (28.1%), followed by GPs (16.1%) and surgery (9.8%) and low proportions in ENT (3.8%), paediatrics (1.6%), and gynaecology (0.9%). The amount of community acquired MRSA cannot be estimated. All disciplines except paediatrics and gynaecology showed an increase over the years. Internal medicine peaked with 36% in 2006.

Conclusions: Analysis of AST data from the Berlin area showed a continuous increase of MRSA proportions in ambulatory care settings within the period from 2000 to 2006 reaching and passing levels known from hospital settings. In the light of these results the ambulatory care sector should be included into a national antimicrobial resistance surveillance system; this will be started in Germany in 2008.

P1736 **Ciprofloxacin resistance in *Staphylococcus aureus*: a 12-year experience in a major Canadian health centre**

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Objective: To observe an evolution of a ciprofloxacin resistance pattern in *Staphylococcus aureus* isolated from a patient population served by a large Canadian hospital over 12 years.
Method: *Staphylococcus aureus* isolated from clinical specimens collected between 1994 and 2005 was used as the representative organism in this retrospective study. Microbiology laboratory database was reviewed for three sentinel years (1994, 1999 and 2005) regarding patient age, gender and specimen source. Organism identification and resistance to ciprofloxacin was determined by either/or: BioMerrieux VITEK, Becton Dickinson Phoenix, Kirby Bauer disk diffusion, AB Biodisk E-test, Becton Dickinson Pasco and/or Dade Behring MicroScan, using resistance criteria from Clinical and Laboratory Standards Institute(CLSI). Repeat specimens of the same sample type collected within one month were excluded from analysis. Microsoft Excel was used for data analysis.
Results: The 5280 pathogenic *S. aureus* strains from 4089 patients were distributed uniformly throughout three sentinel periods of the study. The most common specimen source for all three periods was surface/wound swab, followed by sputum and fluid/aspirate. Overall ciprofloxacin resistance rates were 3.13% for 1994; this was followed by an increase to 8.82% for 1999 and to 17.9% for 2005. Resistance rates were highest in sputum isolates for 1994 (7%), and urine isolates for 1999 and 2005 (13.3 and 31.1% respectively). In every sentinel period, highest resistance rates were seen elderly (over 60 years of age); males with resistant *S. aureus* far outnumbered the females.
Conclusions: Overall ciprofloxacin resistance rates increased over a 12 year surveillance period. The unexpected plateau of this increase between 1999 and 2005 was most likely due to change in prescribing habits caused by new region-wide antibiotic control initiative. In view of popularity of quinolones in clinical practice we advise a targeted approach by restricting these drugs in elderly males to further limit development of resistance.

P1737 **Evaluation of the Etest GRD strip for identification of hGISA**

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Objective: Continued selective pressure from glycopeptide use has led to non-susceptible strains of *Staphylococcus aureus* including hGISA. Infections with hGISA are associated with prolonged bacteraemia and vancomycin (VA) failure. The gold standard for identification of hGISA is population analysis profile – area under the curve assay (PAP-AUC) where the isolate is plated on agar plates with increasing VA concentrations and resulting CFU/mL are plotted versus VA concentration (Wooton et al. J Clin Microbiol.2007;45:329–32.). This method is time consuming and labor intensive and not suitable for use in clinical laboratories. This study compared a new Etest gradient strip (GRD) for detection of hGISA to PAP-AUC and the Etest macromethod (MET) (Walsh et al. J Clin Microbiol. 2001;39:2439–44.).
Methods: 93 clinical hGISA and 25 clinical non-hGISA strains defined by PAP-AUC were tested with GRD strip consisting of a double-sided gradient with VA and teicoplanin (TP), according to the manufacturer's instructions. An inoculum suspension (0.5 McFarland turbidity) was streaked on a Mueller-Hinton (MH) and MH + 5% sheep blood (MHB) agar plates. Etest VA strip was applied to the MH plate and GRD strip to the MHB plate. VA MIC results were read at 24 h and GRD results were read at 24 and 48 h. For GRD, VA or TP results $\geqslant$8 µg/mL and Etest VA MIC of $\leqslant$4 was interpreted as positive for hGISA. MET was done as previously described.
Results: Sensitivity and specifity for GRD was 81% and 96% at 24 h and 93% and 79% at 48 h, respectively and for MET, 76% and 100% at 48 h. For hGISA strains, VA MIC50/90 were 1.5/2 µg/mL and for non-hGISA, corresponding values were 1/1.5.
Conclusion: Etest GRD correctly identified 81% of hGISA at 24 h with high specificity and sensitivity improved to 93% at 48 h. Further evaluation of this test is warranted.

P1738 **Surveillance of *Staphylococcus aureus* susceptibility to fusidic acid in five Canadian laboratories**

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Objective: Fusidic acid has been available on the Canadian market since the early 1980s. The objective of this study was to perform regular surveillance of fusidic acid susceptibility of *Staphylococcus aureus* strains in five Canadian diagnostic laboratories.
Methods: *S. aureus* strains were collected from cultures submitted to the routine laboratory during March and September on an annual basis. Sixty (60) strains were collected from each of the five diagnostic laboratory sites; 10 strains from each site were meticillin resistant *Staph. aureus* (MRSA) isolates. Only one strain per patient was tested in each test period. Routine antimicrobial susceptibility testing was performed in the submitting laboratory by an automated susceptibility method. Testing for fusidic acid susceptibility was performed by disk diffusion (Oxoid, Nepean, Ontario) and ETest® (AB Biodisk, Solna, Sweden) on Mueller Hinton agar plates. The plates were incubated at 35º C for 18–20 hr in ambient air. A zone diameter of >20 mm and an MIC of $\leqslant$2 mg/L (Leo Pharma Inc) were considered to be susceptible.
Results: 603 *S. aureus* strains were tested for fusidic acid susceptibility during 2007, including 100 MRSA strains. 187/603 (31%) of strains were collected from inpatients and 416/603 (69%) from outpatients. 22/300 (7.3%) were resistant to fusidic acid in March and 32/303 (10.6%) in September. 3/50 (6%) strains of MRSA were resistant to fusidic acid in March and 2/50 (4%) in September. All 5 strains of MRSA resistant to FA in 2007 were isolated from one laboratory. Spa typing showed that the fusidic acid resistant strains appeared to be clonal, and not related to resistant clones observed in Europe.
Conclusions: Fusidic acid is the most widely prescribed topical antimicrobial in Canada. Compared to data gathered over the previous 7 years, ongoing surveillance suggests that there has been no trend towards increasing fusidic acid resistance in *S. aureus* strains isolated across the country. The resistance rate remains low even among the MRSA strains submitted for testing. These data support the importance of continuing surveillance and suggest that fusidic acid has maintained its efficacy over the years.

P1739 **Occurrence of fluoroquinolone resistance in members of the *Staphylococcus sciuri* group**

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Objectives: The *Staphylococcus sciuri* group includes *S. sciuri*, *S. lentus*, and *S. vitulinus*. It has been reported that as much as 83% of tested *S. sciuri* isolates exhibit intermediate resistance, and 17% near intermediate resistance to ciprofloxacin. It was suggested that this property may be related to the intrinsic resistance of the organism to novobiocin and nalidixic acid, other antibiotics that inhibit DNA gyrase activity. The aim of this study was to establish the frequency of resistance to fluoroquinolones in large number of the *S. sciuri* group isolates and to test the hypothesis that these bacteria display natural resistance to fluoroquinolones.
Methods: A total of 322 *S. sciuri* group isolates were investigated in this study: 295 *S. sciuri*, 14 *S. lentus*, and 8 *S. vitulinus* strains, and reference strains *S. vitulinus* CCM 4511, *S. lentus* CCM 3472, *S. sciuri* ssp. *sciuri* CCM 3473, *S. sciuri* ssp. *rodentium* CCM 4657, *S. sciuri* ssp. *carnaticus* CCM 4835. The susceptibility to ciprofloxacin, levofloxacin, ofloxacin, norfloxacin, and enoxacin was tested by disk diffusion method in accordance with the recommendations given by Clinical and Laboratory Standards Institute.

Results: Among 322 tested members of the *S. sciuri* group resistance to fluoroquinolones was observed in only 3 isolates (0.9%). The resistance was detected in 2 of 298 (0.7%) *S. sciuri* and 1 of 15 (6.7%) *S. lentus* isolates, while all *S. vitulinus* strains were susceptible to tested fluoroquinolones. One *S. sciuri* strain displaying resistance was isolated from a hospitalised patient, and another from the hospital environment. The PFGE analysis of these isolates showed that they were not related. Resistant *S. lentus* isolate was isolated from an outpatient with frequent urinary tract infections treated with various antibiotics, including fluoroquinolones. Therefore, it seems reasonable to assume that all fluoroquinolone resistant isolates have previously been in contact with fluoroquinolones.
Conclusion: In contrast with previously reported high rate of intermediate resistance to fluoroquinolones in the *S. sciuri* group members, our survey showed low frequency of resistance to these antimicrobials in large series of the *S. sciuri* group isolates. Great majority of the isolates displayed full susceptibility to fluoroquinolones which strongly suggests that members of the *S. sciuri* group are not naturally resistant to these antimicrobials.

P1740 Vancomycin MIC distributions in MRSA isolates from a large acute English hospital: no evidence of MIC shift, 1999–2006

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Objectives: Over the last two decades there has been a steady increase in the use of vancomycin for treatment of severe MRSA infection. Recently concerns have been raised regarding the decreasing efficacy of vancomycin in the clinical setting. In addition there have been conflicting reports of a 'shift' in vancomycin MICs for Staphylococci and Enterococci. In our hospital glycopeptides account for 2.2% of all antibiotic use (upper quartile for SW England) average 5000DDD/annum; >90% of which is vancomycin. The aim of this study was to compare the vancomycin MICs of MRSAs from 1999–2000 and 2006–7.
Methods: A total 396 MRSA isolates from 1999–2000 (197) and 2006–2007 (197) held in the collection at Microbiology Dept, Southmead Hospital were tested. MICs were performed using modified CLSI agar dilution methodology using MIC concentrations of 0.25, 0.5, 1, 1.5, 2 and 4 mg/L.
Results: The range, MIC50 and MIC90 for 1999–2000 and 2000–2007 were equivalent <0.25–1.5, 1 and 1 mg/L respectively. The modal MIC for both sets was 1 mg/L. All isolates were inhibited by vancomycin 2 mg/L.

MIC	No. of isolates	
	1999–2000 (n = 197)	2006–2007 (n = 197)
⩽0.25	2	1
0.5	49	51
1	144	144
1.5	2	1
2	0	0
4	0	0

Conclusions: Vancomycin shift was not observed over the 6 year period tested despite high vancomycin usage. Vancomycin remains a viable option for the treatment of MRSA infection in our hospital.

P1741 In vitro susceptibility of group B *Streptococcus* strains isolated from genital tract of pregnant women

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Objectives: Group B *Streptococcus* (GBS) early neonatal sepsis prevention, requires GBS detection in pregnant women genital tract, and intrapartum prophylaxis with penicillin or ampicillin administration. In women with allergy to penicillin, administration of clindamycin or erythromycin, and vancomycin in case of resistance to these antibiotics, is suggested. The aim of the study is the GBS resistance surveillance.
Methods: Between May to November 2007, in six different Microbiology laboratories of Barcelona, Spain, the in vitro susceptibility of 591 GBS strains was studied. The strains were isolated from genital tract (vaginal and/or rectal swabs) of women between 35–37 weeks of gestation. Susceptibility to clindamycin, erythromycin, telithromycin and fosfomycin was performed by disk-diffusion and/or microdilution tests. The resistance mechanisms to clindamycin, erythromycin and telithromycin were assessed by disk-diffusion method.
Results: 140 strains (236%) showed resistance to any of four antibiotics: 102 (17.2%) to clindamycin, 106 (17.9%) to erythromycin, 18 (3.0%) to telithromycin and 29 (4.9%) to fosfomycin.
86 strains presented a constitutive MLS-B phenotype (clindamycin and erythromycin resistant), 14 inducible MLS-B phenotype (erythromycin resistant and clindamycin inducible resistance), 6 M phenotype (erythromycin resistant and clindamycin susceptible) and 2, unknown mechanism (erythromycin susceptible and clindamycin resistant). Of the 18 strains with resistance to telithromycin, 13 presented a constitutive phenotype and 5 an inducible phenotype.
Increasing resistance rate to these antibiotics has been observed since the last multicentre study performed in Spain in year 2002 (with resistance rates as follows: 12.4% to erythromycin, 11.8% to clindamycin, 1.8% to telithromycin and 0.3% to fosfomycin).
Conclusion: Susceptibility surveillance is required in genital GBS strains isolated from penicillin allergic women, due to the high and increasing resistance rates to clindamycin and erythromycin. Since GBS remains quite susceptible to fosfomycin, this antibiotic could be an alternative in some cases.

P1742 Serotyping and antimicrobial resistance of group B *Streptococcus* in Korea

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Purpose: Because of the prevalence of Group B *Streptococcus* (*Streptococcus agalactiae*, GBS) among pregnant women and disease burdens in neonates and adults are increasing in Korea; we carried out this molecular epidemiologic study on GBS to estimate the serotype distribution and antimicrobial resistance among colonising and invasive isolates.
Materials and Methods: Colonising isolates (199) were collected by GBS screening in pregnant women (35–37 weeks) between Nov. 2004 and Mar. 2007 in four hospitals of Seoul and Daejeon. Invasive isolates (243) came from patients of clinics or hospitals in whole country between Feb. 2006 and Mar. 2007. To confirm serotype of GBS, co-agglutination assay (serotyping kit) and microarray methods were used. To test antimicrobial resistance, disc diffusion method for colonising isolates and MIC for invasive isolates were used.
Results: Serotype III was predominant and V was the next frequent in colonising isolates, but serotype V was predominant and III was the next in invasive isolates. Antimicrobial resistances for colonising and invasive isolates were high; resistance to erythromycin (27.9%, 35.3%, respectively) and resistance to clindamycin (46.3%, 48.7%, respectively). We also confirmed the resistance to cefazolin and cefuroxime at first

in Korea. Antimicrobial resistance was high in serotype V, VII and VIII, and especially, resistance to clindamycin rather than erythromycin was very high in serotype III. When the isolates showed resistance to erythromycin, they also showed resistance to clindamycin except two isolates. However, when the isolates had resistance to clindamycin, 31.7% of them (almost of all was serotype III) were susceptible to erythromycin, which were very different characteristics of Korean GBS isolates.

Conclusion: Serotypes of Korean GBS isolates were moved to virulent serotype V, VII, and VIII and antimicrobial resistance to erythromycin and clindamycin were still increasing: further research and control on GBS were very important in Korea.

P1743 Antimicrobial susceptibility profiles of group B streptococci in 2006–2007, including one unusual penicillin non-susceptible strain

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Objectives: We have determined the antimicrobial susceptibilities of group B streptococci (GBS) strains isolated in our laboratory from clinical specimens to explore the penicillin MIC values for which the organism is considered to be susceptible and investigated inducible clindamycin resistance among these strains.

Methods: A total of 158 GBS have been isolated in our laboratory between 2006 and 2007. Identification has been performed using latex agglutination test (Pastorex Strep B, Bio-Rad). For all the strains penicillin MIC values have been established with Etest strips (following manufacturer's instructions) and disc diffusion test for erythromycin, clindamycin and tetracycline according to CLSI. Erythromycin and clindamycin discs have been placed 25 mm apart from each other (centre to centre) to observe the inducible clindamycin resistance. A blunting in the inhibition zone of clindamycin facing the erythromycin disc has been considered positive for inducible clindamycin resistance.

Results: Tetracycline, erythromycin and clindamycin resistance has been found as 94.3%, 34.2% and 29.7%, respectively. Inducible clindamycin resistance has been observed in 3 (1.9%) of the strains. Penicillin MIC50 and MIC90 values have been found as 0.047 ug/ml and 0.064 ug/ml, respectively. Excluding one GBS strain with penicillin MIC of 1 ug/ml, penicillin MIC values have been found in susceptibility limits with the highest penicillin MIC value being 0.094 ug/ml.

Conclusion: The penicillin nonsusceptible results for GBS have been reported in the last years from different geographic areas. In this study we have detected one penicillin nonsusceptible GBS strain with penicillin MIC of 1 ug/ml. This strain isolated from a patient's urine indicates that penicillin susceptibility of GBS strains should be monitored carefully.

P1744 Antimicrobial susceptibilities of *Streptococcus pyogenes* isolated from respiratory and skin infections in the United States in 2005–2006

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Objective: *Streptococcus pyogenes* (SP) causes a variety of diseases ranging from non-invasive mild infections such as pharyngitis and impetigo to severe invasive infections such as bacteraemia, pneumonia and necrotising fasciitis. Macrolides are widely used to treat SP infections in subjects with known allergies to β-lactams. Macrolide-resistant SP has been reported and continues to emerge in the U.S. The aim of the study was to assess the prevalence of resistant phenotypes of SP collected in the U.S. in the 2005–2006 Faropenem surveillance study.

Methods: 1001 SP were collected from 104 centres in the USA during 2005–2006 and tested against azithromycin (AZI), cefdinir (CDN), cefuroxime (CFX), co-amoxyclav (AMC), co-trimoxazole (TMP-SMX), faropenem (FAR), levofloxacin (LEV), penicillin (PEN), telithromycin (TEL) by broth microdilution according to CLSI guidelines. AZI resistance (AZI-R) was determined using CLSI breakpoints and results analysed according to geographic and demographic information.

Results: AZI-R was 7% for all U.S. isolates and varied by region ranging from 3.9% in south central to 9.3% in the northeast. The MIC90s for AZI ranged from 0.25 mg/L for susceptible (S) strains to >16 mg/L for AZI-R strains. AZI-R varied with age of subject ranging from 12.9% (<2 yrs) to 0% (>65 yrs), and specimen source with R rates of 3.7, 7 and 9.4% respectively in blood, skin and respiratory isolates. AZI-S and AZI-R SP were equally susceptible to all β-lactams tested including FAR (MIC90, 0.03 mg/L). In contrast, TEL was less active against AZI-R strains (MIC90, 8 mg/L) when compared with AZI-S SP (MIC90, ⩽0.12 mg/L). All isolates were inhibited by LEV at concentrations ⩽2 mg/L.

Conclusions: AZI-R SP remained prevalent in the U.S. in 2005–2006 especially in isolates from children ⩽2 yrs old. FAR, other β-lactams and TMP-SMX retain activity against AZI-S-and AZI-R phenotypes.

P1745 Activity of faropenem against U.S. paediatric isolates of serotype 19A *Streptococcus pneumoniae* collected in the 2005–2006 respiratory season

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Objective: *Streptococcus pneumoniae* (SP) is the leading cause of bacterial pneumonia, sinusitis and acute otitis media in children worldwide. The heptavalent pneumococcal vaccine (PCV7) was considered to be a promising strategy for reducing invasive pneumococcal infections in children following its introduction in the U.S. in 2000. However, increasing antibiotic resistance is now being observed in the U.S. especially among serotypes of SP not covered by PCV7 such as multidrug-resistant serotype 19A strains. The aim of the study was to assess the serotype distribution and antimicrobial susceptibility of SP collected from U.S. children in 2005–2006.

Methods: 393 SP were collected from children at 104 U.S. centres; 307 (<5 yrs), 86 (6–14 yrs). Specimen sources included 199 invasive, 119 non-invasive and 74 carriage sites. Serotyping was performed by quellung reaction with type-specific antisera. MICs of azithromycin (AZI), cefdinir (CDN), cefuroxime (CFX), co-amoxyclav (AMC), co-trimoxazole (TMP-SMX), faropenem (FAR), levofloxacin (LEV), penicillin (PEN) and telithromycin (TEL) were determined and interpreted according to CLSI guidelines.

Results: Serotype 19A was the most frequent type, accounting for 30.5% of isolates, followed by serotypes 6A, 3, 19F, 35B, 23A and 15A with prevalence ranging from 9.9% to 3.6%. FAR was the most potent β-lactam against serotype 19A strains (MIC90 1 mg/L) compared with AMC, CDN and CFX (MIC90s of 8, >8 and 16 mg/L, respectively, with >35% of isolates nonsusceptible to these agents). Serotype 19A strains also had reduced susceptibility to AZI and TMP-SMX with MIC90s of >16 and >8 mg/L, and 64% and 73% of isolates resistant, respectively. All serotype 19A strains were susceptible to LEV and TEL, agents unlikely to be widely used to treat paediatric infections in children because of safety concerns.

Conclusions: Serotype 19A and other non-vaccine serotypes of SP were more prevalent than the vaccine serotypes covered by PCV7 in 2005–2006. FAR was the most potent β-lactam against SP serotype 19A isolates and was 8-fold more active than AMC. Further studies are warranted to evaluate clinical efficacy of FAR against infections caused by serotype 19A isolates.

P1746 Prevalence of low-level and high-level resistance to fluoroquinolones in *Streptococcus pneumoniae* in Getafe, Spain

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Fluoroquinolone (FQ) resistance among strains of *Streptococcus pneumoniae* has emerged worlwide. Strains with high-level resistance and even with low-level resistance (first-step mutants) have been associated with treatment failures.

Objectives: The aim of this study was to assess the prevalence of low-level and high-level resistance to fluoroquinolones in *Streptococcus pneumoniae* strains of Getafe, Spain.

Methods: A total of 100 *S. pneumoniae* strains (28 from children and 72 from adults) were isolated from clinical samples between November 2006 and June 2007 at the Department of Clinical Microbiology of Getafe University Hospital. The main sources of the isolates were blood samples (42%), conjunctival exudates (17%), and respiratory samples (37%).
Susceptibility to 5 FQ (levofloxacin, norfloxacin, pefloxacin, ciprofloxacin, and sparfloxacin) was performed using disk diffusion method. The results were interpreted following the phenotypic test designed for Varon et al. (Antimicrob Agents Chemother 2006;50:572–9) for the detection of the low- and high-level FQ-resistant mutants according to the inhibition zone diameters of the 5 FQ.
We also performed the broth microdilution method to determine the susceptibility to levofloxacin.
Results: 4 of the isolates showed resistance to levofloxacin (high-level resistant mutants) and 96 were susceptible by the broth microdilution method and CLSI guidelines. Using the FQ disks and the interpretations of Varon et al. 60 strains had the wild phenotype (susceptible), 5 strains had an increased efflux and the rest harboured a phenotype compatible with topoisomerase IV (28 strains) or gyrase (3 strains) mutations.
Conclusions: The results of the study showed that 34.5% of the strains susceptible to levofloxacin by the CLSI had a low-level resistance to this antibiotic and so the potencial to generate high level resistant mutants.

P1747 Epidemiological survey of resistance to β-lactams (AMX, CFX, CRO), macrolides (CLR, TEL), and fluoroquinolones (LVX, MXF) in a Belgian collection of community-acquired pneumonia isolates of *Streptococcus pneumoniae*

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Objectives: Evaluate current resistance trends in *Streptococcus pneumoniae* (SP) (including efflux) from CAP patients towards the main antibiotics registered for this indication.
Methods: 142 SP isolated in 4 major teaching hospitals over the last 3 years from patients admitted from the community and with CAP diagnosis confirmed by retrospective analysis of medical records. MIC determined by microdilution in CAMH broth + 2.5% horse blood. Susceptibility assessed according to EUCAST and CLSI bkpts.
Results: Based on CLSI bkpt, 99.3% of isolates remained susceptible to AMX, but MIC90 values are elevated. Non-susceptibility to CFX and CRO was observed in about 15% and 10% of isolates, respectively, whatever the bkpt considered. More than 34% of isolates were resistant to CLR, among which 20% [7% of the collection] remained susceptible to clindamycin (denoting efflux). 7% of isolates could be categorised as TEL-I or -R using EUCAST bkpts but only 1.4% would appear as TEL-I according to CLSI. MICs of LVX were higher than those of MXF, but non-susceptibility to both drugs remained low (due to higher LVX bkpts). Reserpine had only a modest effect (0.7% remaining LVX-R according to EUCAST, no change for MXF).

AB	MIC_{50}	MIC_{90}	Range		EUCAST[a]		CLSI[b]	
			min	max	% I	% R	% I	% R
Amoxycillin [AMX]	0.06	1.5	⩽0.03	16			0	0.7
Cefuroxime [CFX]	0.06	8	⩽0.03	16	2.1	13.4	2.8	12.7
Ceftriaxone [CRO]	0.06	1.5	⩽0.016	3	11.3	0.7	10.6	0
Clarithromycin [CLR]	⩽0.06	>32	⩽0.06	>32	0	35.2	0.7	34.5
Telithromycin [TEL]	⩽0.06	0.25	⩽0.06	2	4.2	2.8	1.4	0
Levofloxacin [LVX]	0.75	1.5	0.375	12	0	2.1	1.4	0.7
Moxifloxacin [MXF]	0.25	0.25	0.05	3	0	0.7	0.7	0

EUCAST bkpts: AMX: none proposed; CFX (published): S ⩽ 0.5, R > 1; CRO (published): S ⩽ 0.5, R > 2; CLR (proposed): S ⩽ 0.25, R > 0.5; TEL (proposed): S ⩽ 0.25, R > 0.5; LVX (published): S ⩽ 2 < R; MXF (published): S ⩽ 0.5 < R.
[b]CLSI bkpts: AMX: S ⩽ 2, R ⩾ 8; CFX: S ⩽ 0.5, R ⩾ 2; CRO: S ⩽ 1, R ⩾ 4; CLR: S ⩽ 0.25, R ⩾ 1; TEL: S ⩽ 1, R ⩾ 4; LVX: S ⩽ 2, R ⩾ 8; MXF: S ⩽ 1, R ⩾ 4.

Conclusion: The data show a marked decreased susceptibility of SP to cephalosporins, and a high prevalence of MLSB resistance. Resistance to TEL and quinolones remains negligible based on CLSI breakpoints, but become noticeable for TEL if using EUCAST proposed bkpt. LVX MICs are clearly increased. Efflux significantly impacts on macrolide susceptibility but does not markedly affect LVX or MXF. The data (i) underline the risk of empirical treatment of CAP with cephalosporins and macrolides, (ii) justify the high dose approach with AMX; and (iii) illustrate the importance of setting appropriate bkpts.

P1748 Trends in nasopharyngeal carriage of *Streptococcus pneumoniae* among children attending daycare centres in France: 1999–2006

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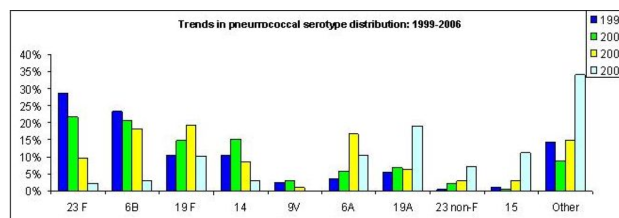
Objectives: To assess trends in pneumococcal carriage among children attending day-care centres (DCC) in France following implementation of pneumococcal conjugate vaccine (Pn7) and interventions promoting prudent antibiotic use.
Method: Cross-sectional surveys were performed in the Alpes Maritimes (AM) and Nord (ND) areas between January and March 1999, 2002, 2004 and 2006 on a random sample of children attending group day-care. A local intervention promoting prudent antibiotic use in paediatric respiratory tract infections was conducted in AM in 2000 and 2003, via academic detailing visits to all general practitioners and paediatricians, and a national media campaign began in 2002. Pn7 became available in 2002. For each study period, antibiotic susceptibility and serotype distribution of isolates from nasopharyngeal aspirates were recorded, as well as immunisation status.
Results: Nasopharyngeal carriage of SP remained stable in AM: 161/298 (54.0%) samples in 1999 and 168/335 in 2006; and in ND: 117/250 (46.8%) samples in 1999 and 146/288 (50.7%) in 2006, while the proportion of SP with diminished susceptibility to penicillin (PDSP) decreased significantly in both areas between 1999 and 2006, from 101/161 (62.7%) to 57/168 (33.9%) in AM ($p < 10^{-3}$), and from 84/117 (71.8%) to 82/146 (56.2%) in ND ($p < 10^{-3}$). Antibiotic use as reported by parents dropped significantly in both areas, by 50% in AM and by 25% in ND. Overall susceptibility of strains to antibiotics increased. Over two-thirds of the children had received at least one vaccine dose in 2006. A shift from Pn7 serotypes (6B, 9V, 19F, 23F) towards Pn7 cross-reactive (6A, 19A, 23 non-F) and non-vaccine types was observed (Figure 1).



Pooling both areas, among PDSP, serotype 6A, 19A, 19F and 15 together accounted for 75% of strains in 2006 versus 16% in 1999 ($p < 10^5$).
Conclusion: The distribution of nasopharyngeal carriage of SP has changed since the introduction of Pn7 in favour of vaccine-related and of non-vaccine strains with a significant increase in antibiotic susceptibility; however the proportion of non-vaccine types has increased among PDSP. Replacement strains must be protected from selective pressure resulting from inappropriate antibiotic use in order to uphold the benefit provided by the vaccine.

P1749 In vitro susceptibility of respiratory isolates of *Streptococcus pneumoniae* and *Streptococcus pyogenes* in a twelve-month period

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Therapy is often empirical in respiratory tract infections and there is a need for local data on the rate of resistance to available antimicrobials. Increased levels of macrolide-resistant *Streptococcus pyogenes* and *Streptococcus pneumoniae* in several regions have been reported.

Objective: To study the antimicrobial susceptibility of two major pathogens: *Streptococcus pneumoniae* and *Streptococcus pyogenes*, to define the place of several alternative regimens in the first line therapy for pharyngitis and lower respiratory tract infection.

Methods: Antimicrobial susceptibility tests analysis for a total of 232 unique isolates of *Streptococcus pyogenes* and 107 isolates of *Streptococcus pneumoniae* recovered between December 2006 and November 2007 at the National Matei Bals Infectious Diseases Institute, Bucharest, as a part of the MART surveillance study. Antibiotic susceptibilities were determined with disk diffusion method and confirmed with E-tests for penicillin.

Results: *S. pyogenes* isolates were uniformly susceptible to β-lactams, sulfamethoxazole/trimethoprim and vancomycin; macrolide-resistance was noted for 6.55% of strains (9 strains with M phenotype and 6 strains with MLSB resistance). Three isolates were resistant to levofloxacin (1.4%), all macrolide-susceptible, and six isolates resistant to tetracycline (5.3%), two of them macrolide-resistant ones. *S. pneumoniae* strains were uniformly susceptible for moxifloxacin, vancomycin and linezolid; diminished susceptibility to penicillin was noted for 57.1% strains, macrolide-resistance for 47.2% strains (two thirds with MLSB phenotype) and multiresistance for 43.9% strains. Susceptibility of penicillin-resistant strains was: 96.3% for rifampin, 85.5% for cloramphenicol, 80% for tetracycline, 44% for clindamycin. Susceptibility of macrolide resistant strains were: rifampine 98.7%, ceftriaxone 62.6%, tetracycline 21%.

Conclusion: Macrolide-resistance is yet at low level for *S. pyogenes*, but higher for *S. pneumoniae*, probably due to increased consumption of macrolides. The risk of selective pressure with continuous rise of macrolide-resistance whatever it is the indication for usage, could impose a restricted access, especially in outpatient pharyngitis or respiratory infections. A similar situation could be defined for sulfamethoxazole/trimethoprim; concerning doxycycline, is possible that breakpoints are too high and/or defined daily dose too low for pneumococcal infections.

P1750 Global trends of penicillin resistance rates in *Streptococcus pneumoniae*, 2004–2007

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Objectives: The percentage rates of penicillin-resistant (PenR) *S. pneumoniae* (SPN) vary by country and region. The purpose of this analysis was to determine changes in regional variations of PenR and PenNS strains of SPN, and the current activity of tigecycline (TIG), amoxicillin-clavulanic acid (AC), ceftriaxone (CFX), levofloxacin (LEV), linezolid (LNZ), and vancomycin (VAN) against pen-resistant isolates.

Methods: 5,010 clinically relevant isolates of SPN were collected from patients in 387 hospitals worldwide from 2004 to 2007. MIC's to all agents tested were determined by broth microdilution and interpreted following CLSI guidelines.

Results: Number of SPN and the percentage non-susceptible to penicillin from 2004–2007 are summarised in the table.

% of PenR SPN susceptible to AC ranged from 34–100; CFX from 33–100. 100% of PenR SPN were susceptible to TIG, LEV, LNZ, and VAN.

Conclusions: Rates of PenNS for SPN range from 29% in Europe to 59% in South Africa. Magnitude of changes from 2004 to 2007 varied by region, being fairly constant in Europe, Middle East, and North America, but showing swings ranging from 10–31% (Latin America and Asia/Pacific Rim, respectively). Generally speaking, PenNS rates were essentially constant from 2006–2007; however, the advent of new higher CLSI breakpoints for intravenous penicillin for non-meningitis SPN in 2008 will lead to significant declines in reported PenNS rates in countries using CLSI standards. Utility of AC and CFX vs. PenR SPN varies widely by region, and local susceptibility patterns must be taken into account when selecting therapy.

Region	Number of strains (%R+I)			
	2004	2005	2006	2007
South Africa	–	19 (58%)	47 (73%)	22 (59%)
Asia/Pacific Rim	22 (18%)	43 (35%)	120 (49%)	147 (42%)
Europe	220 (28%)	208 (28%)	510 (29%)	325 (29%)
Latin America	–	63 (29%)	146 (37%)	84 (39%)
Middle East	–	10 (40%)	11 (36%)	14 (43%)
North America	447 (45%)	741 (42%)	1213 (43%)	598 (37%)

P1751 Trends in antimicrobial resistance in *S. pneumoniae*, Canada, 1993–2007

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Background: The Canadian Bacterial Surveillance Network (CBSN) has been monitoring resistance trends in Canadian isolates of *S. pneumoniae* (SP) since 1993.

Methods: CBSN is a collaborative network of microbiology laboratories from across Canada that submit bacterial isolates to a central laboratory for standardised antimicrobial susceptibility testing performed according to CLSI protocols.

Results: Of the 28,915 SP isolates submitted and tested between 1993 and July of 2007, 37% were from blood/CSF, 41% from sputum, 22% from other sites. The trends in antimicrobial susceptibility are expressed below as percentage resistant. Macrolide resistance and tetracycline resistance have both increased slightly since 2006 (19.2 vs 21.7 and 11.3 vs 12.3 respectively). Resistance to fluoroquinolones declined slightly since 2006 (in 2007: Cip 1.7%; Levo 0.8%; Moxi 0.5%). Rates of resistance to β-lactams were increased in 2006 compared to previous years but penicillin resistance decreased in 2007 from to 6.2% in 2006 to 4.1%, P = 0.009). Ceftriaxone resistance (meningeal breakpoint MIC ⩾2) decreased slightly from 3.7% to 2.5, P=NS. Amoxicillin resistance increased significantly from 0.1% in 2000 to 1.4% in 2006, and 1.3% in 2007.

	’93	’94	’95	’96	’97	’98	’99	’00	’01	’02	’03	’04	’05	’06	’07
Pen NS	5.7	8.1	8.8	12.1	13.6	15.1	13.5	12.4	14.5	15.0	14.9	14.8	15.3	15.2	15.9
Pen R	0.9	1.3	2.2	4.2	6.6	5.7	5.9	5.9	6.9	6.5	6.3	5.4	4.8	6.2	4.1
Amox R	0	0	0	0	0.1	0.1	0.2	0.1	0.5	0.6	1.2	1.1	1.1	1.4	1.3
Eryth R	1.9	3.4	3.2	5.1	6.8	10.6	9.9	11.2	12.9	14.2	16.1	18.	19.1	19.2	21.7
Clinda R	0	1.7	1.3	2.5	3.6	5.2	4.4	5.5	5.8	6.6	7.3	8.1	8.2	8.6	8.4
T/S R	3.8	4.7	9.7	12.6	14.6	12.3	12.0	11.3	11.9	13.1	13.4	13.4	12.2	11.8	10.6
Tet R	1.4	2.3	3.4	2.5	6.4	9.1	7.1	5.5	9.1	9.9	9.8	10.9	10.4	11.3	12.3
Ceftrx R(M)	0	0.2	0.1	0.7	1.3	2.5	1.5	2.0	2.4	1.5	1.8	2.5	1.9	3.7	2.5
Ceftrx R(NM)	0	0.4	0	0.2	0.1	0.1	0.3	0.1	0.2	0.2	0.1	0.1	0.2	0.2	0.2
Cipro R	0.5	0.8	0.7	0.8	1.8	1.8	1.7	1.4	2.4	2.7	1.6	2.1	2.4	2.7	1.7
Levo R	0	0.4	0.1	0.2	0.5	0.3	0.4	0.9	1.3	1.9	1.1	1.5	1.5	1.7	0.8
Moxi R	NT	NT	0	0	0.3	0.2	0.3	0.4	0.3	0.3	0.3	0.6	0.7	0.9	0.5

Conclusions: We continue to see an increasing trend in rates of macrolide resistance in Canada. The significant increase in β-lactam resistance recognised in 2006 has stabilised in 2007. Fluoroquinolone resistance appears to be declining.

P1752 European survey of macrolide resistance mechanisms among *S. pneumoniae* from 2006–2007 and activity of telithromycin

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Objectives: To determine the activity of telithromycin against macrolide-resistant strains of *Streptococcus pneumoniae* from patients with community-acquired respiratory tract infections (RTIs).

Methods: Isolates were collected between January 2006 and April 2007 in 17 European countries (156 centres) from patients with community-acquired RTIs either in the community or from those hospitalised for less than 48 hours. In vitro antibacterial activity and susceptibilities of *S. pneumoniae* isolates were determined at a central laboratory using Clinical and Laboratory Standards Institute methodology and interpretive criteria. Macrolide resistance genotypes were determined by PCR.

Results: Of the 3026 *S. pneumoniae* isolates collected, 26.0% were resistant to macrolides. Macrolide resistance rates varied widely between countries, ranging from 3.8% in the Netherlands to 52.2% in Italy. High rates of resistance (>20%) were observed in Belgium, Finland, France, Hungary, Italy, Poland, the Slovak Republic and Spain. Overall, the most common macrolide resistance mechanism was erm(B) which was found in 19.3% of all isolates (74.4% of erythromycin-resistant *S. pneumoniae* [ERSP]), while the efflux gene mef(A) was found in 5.6% of all isolates (21.2% of ERSP). erm(B) and mef(A) mechanisms were found together in 0.5% of all isolates (2.0% of ERSP). The erm(B) gene was the predominant mechanism in all countries except Austria and the UK, where efflux was more prevalent than erm(B). Overall, 99.2% of *S. pneumoniae* isolates were susceptible to telithromycin. The susceptibility rates for telithromycin in macrolide-resistant isolates were 95.9% (erm[B]), 99.4% (mef[A]), 100% (erm[B] + mef[A]) and 100% (ribosomal mutations).

Conclusions: Macrolide resistance remains high across Europe, with erm(B) being the most common form of resistance. This surveillance study confirms that telithromycin is active against macrolide-resistant isolates of *S. pneumoniae*, irrespective of the macrolide resistance mechanism.

P1753 Pneumococcal invasive diseases in Slovenia, 2004–2007

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Objectives: *Streptococcus pneumoniae* (SP) is one of the most frequent cause of bacterial invasive diseases in children and elderly. In Slovenia the epidemiologic situation of invasive pneumococcal diseases has been constantly monitored since 1993 in order to formulate optimal vaccination strategy.

Methods: Isolates of *S. pneumoniae*, isolated from blood or CSF of patients with invasive diseases, were nationally collected. All isolates were typed with capsular swelling reaction. Antibiotic susceptibility was determined by disc diffusion test and E-test following the CLSI recommendations. The results were also interpreted according to the criteria from the NCCLS standards.

Results: From the year 2004 to October 2007 a total of 685 invasive isolates of SP were collected. The incidence of pneumococcal invasive infections was stable (~9.7/100.000), except in the year 2007, where we observed the increase in incidence among children (from 10.3 in 2004 to 17.8* in 2007). Total resistance by year is presented in Table 1. The most common serotypes in children were 14, 1, 6B, 4, 23F, 19F and 18C, in adults 3, 1, 14, 4, 9V, 7F and 23F. The most prevalent serotypes among penicillin resistant isolates in children were 14 and 9V (76.9%), in adults 14, 9V, 19A and 19F (75.3%). We detected also 9 multiresistant isolates (PEN, ERY, TMP/SMX) in children (5.5%) and 10 isolates (1.9%) in adults.

Conclusions: The patterns of resistance and serotypes indicate the presence of multiresistant clones of SP in Slovenia. To determine these clones molecular typing has to be done. Nevertheless the prevalent multiresistant isolates belong to serotypes which are included in 7-valent conjugated pneumococcal vaccine.

Table 1: Percentage of resistance (I+R) in invasive *S. pneumoniae* in Slovenia, 2004–2007*

Year	No.	PEN	CTX (mening)	ERY	TET	TMP/SMX	CHL
2004	171	27.5	11.7	11.7	7.0	27.0	0
2005	211	12.8	6.2	12.3	6.2	12.3	1.3
2006	155	20.6	5.8	13.5	7.7	18.8	0
2007*	148*	18.9*	4.1*	18.9*	10.8*	20.9*	2.0*

*Data till October 2007. PEN – penicillin, ERY – erythromycin, CTX – cephotaxime, TET – tetracycline, TMP/SMX – trimethoprim/sulfamethoxazole, CHL – chloramphenicol.

P1754 Impact of PCV7 on invasive pneumococcal disease in Scotland

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Objectives: In September 2006, the 7-valent pneumococcal conjugate vaccine (PCV7) was introduced into the Scottish Routine Childhood Immunisation Programme with a catch-up campaign to two years of age. There is a concern that there will be an emergence of serotypes not contained in the 7-valent vaccine.

This study evaluated the impact of the 7-valent pneumococcal conjugate vaccine on invasive pneumococcal disease (IPD) in Scotland.

Methods: Health Protection Scotland has routinely collected data on IPD as part of the European Antimicrobial Resistance Surveillance System (EARSS) since 2003.

Data collected between October 2004 and September 2007 inclusive were analysed for this study. Serotype distribution and antimicrobial resistance was investigated for the following age groups:

- <2 years
- 2–65 years
- >65 years

Results: The total number of cases of IPD in Scotland has decreased since the introduction of the PCV7 into the Scottish Routine Childhood Immunisation Programme.

The number of cases in the under two year old population has statistically significantly reduced.

A reduction in the number of cases can also be seen in the over 65 year population.

There has been an increase in some serotypes not covered by the vaccine. There were a significant number of emergent cases of serotype 35B in the under two year old population and a significant increase in the numbers of serotype 7F was observed in the sixty five and over population.

Table 1 shows that susceptibility of *Streptococcus pneumoniae* to penicillin, erthromycin and ciprofloxacin has increased since the introduction of PCV7.

Table 1 Antimicrobial susceptibility of *Streptococcus pneumoniae*

	Sep 2004 to Aug 2005	Sep 2005 to Aug 2006	Sep 2006 to Aug 2007
No. of isolates	486	643	646
% penicillin non-susceptible	2.9%	3.3%	1.9%
% erythromycin non-susceptible	11.3%	12.6%	4.2%
% ciprofloxacin non-susceptible	2.1%	7.5%	4.0%

Conclusion: The introduction of PCV7 into the routine childhood vaccination schedule in Scotland has resulted in a decrease of the incidence of vaccine-serotype IPD, and consequently a fall in the overall incidence of IPD. A decline in IPD incidence in all age groups, including those not targeted in the vaccination programme has also been observed.

This suggests a strong herd community. There has been an increase in susceptibility to penicillin, erythromycin and ciprofloxacin. There has however been an increase in the numbers of serotypes not covered by the vaccine which highlights the fact that continued surveillance of IPD is essential.

P1755 Prevalence of fluoroquinolone resistance and QRDR mutations in *S. pneumoniae* in Toronto, Canada, 1995–2006

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Background: The emergence of fluoroquinolone resistance (FQR) in *S. pneumoniae* (SP) is a concern. We examined FQ use, FQR and QRDR mutations in pneumococci in Toronto, Canada.

Methods: From 1995–2006, the Toronto Invasive Bacterial Diseases Network (TIBDN) collected all sterile site isolates of pneumococci isolated from residents of Toronto/Peel, CA (pop 4M); from 2002–2006, all respiratory tract isolates were also collected. Broth microdilution susceptibility testing to CLSI standards is performed on all isolates, sequencing of QRDR regions was performed on all FQR isolates, and a representative sample of FQ susceptible isolates. Popn FQ use was obtained from IMS Health Canada. Demographic and medical data were collected for all patients.

Results: From 1995 to 2006, overall FQ use increased from 67 to 94 scripts/1000pop/yr; levofloxacin use increased from 0 to 12.6 scripts/1000pop/yr, and moxifloxacin use increased from 0 to 15.3 scripsts/1000 population per year. 7038/7909 (89%) of case isolates were available for testing. Ciprofloxacin non-susceptibility rates increased from 0.9% in 1995 to 4.1% in 2002, then decreased to 2.7% in 2006. Levo R rates increased from 0 in 1995 to 3.0% in 2002, then decreased to 1.8% in 2006. Moxi R rates have increased slowly, to 1.3% in 2005, then decreased to 1.1% in 2006. In cipro susceptible isolates (MIC < 4), the prevalence of parC mutations was 18/1053 (1.7%) and the prevalence of gyrA mutations was 1/1053 (0.09%). The prevalence of parC and gyrA mutations in isolates with levo MIC < 2ug/ml was 0.6% (18/3058) and 0.03%.(1/3058). The prevalence of parC and gyrA mutations in isolated with moxi MIC < 0.25ug/ml was 0.5% (14/2952) and 0 (0/2952). There was no increase over time in the prevalence of mutations in isolates susceptible to FQ. Isolates mutations in gyrA but not in parC were first detected in 2002 (a single isolate), and remain rare (2 isolates in 2006).

Conclusions: Despite increasing use of FQ antibiotics FQR is stable or decreasing in Toronto. Isolates with mutations in gyrA and parC remain very rare in FQ susceptible isolates, Similarly, mutations in gyrA alone remain exceedingly uncommon, suggesting the moxifloxacin exerts minimal selective pressure for resistance.

MRSA detection and infection control

P1756 Clinical comparison fo the molecular-based BD GeneOhm Cdiff assay to the cytotoxicity tissue culture assay for the direct detection of toxin B gene from toxigenic *Clostridium difficile* strains in faecal specimens

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Objectives: The primary objective of this prospective evaluation was to demonstrate the use of the BD GeneOhm™ Cdiff (BD Diagnostics, San Diego, CA) real-time Polymerase Chain Reaction (PCR) assay as a diagnostic test for the detection of toxigenic *Clostridium difficile* (Cdiff) strains from faecal specimens. The performance of PCR was compared to a cytotoxicity reference standard (TechLab®, Blacksburg, VA). The BD GeneOhm™ Cdiff assay is a qualitative in vitro diagnostic test performed on the Cepheid SmartCycler® (Cepheid, Sunnyvale, CA), a random-access real-time PCR instrument. The assay utilizes PCR for the amplification of the toxin B gene (tcdB) and fluorogenic target-specific hybridisation probes for the detection of the amplified DNA. The amplification, detection and interpretation of the signals are done automatically by the SmartCycler® software.

Methods: Liquid to soft stools received in the clinical laboratory for Cdiff testing were included in the evaluation. Briefly, stools were tested with the TechLab Cdiff chek™-60 enzyme immunoassay (EIA) for detection of the “common antigen”, glutamate dehydrogenase (GDH), and positive results were confirmed with the Tox A/B assay. If the Tox A/B was negative, a cytotoxicity neutralisation assay was also performed. Concurrently, the BD GeneOhm™ PCR assay was also performed on each stool specimen. Additional test methods (culture, cytotoxin/neutralisation testing) were also performed on discordant specimens to aid in resolving discrepancies. Each stool was collected, processed, and tested according to the institution's standard of care and each assay was performed according to manufacturer's package insert.

Results: Of the 298 specimens included in this study, 247 (83%) tested negative with both PCR and cytotoxicity while 29 (10%) tested positive with both assays yielding 90.6% sensitivity and 92.9% specificity. After resolution of discordant results, the sensitivity and specificity was 93.6% and 98.4% respectively with a prevalence of nearly 15%.

Conclusions: The diagnosis of toxigenic *C. difficile* is usually done by a combination of cytotoxicity assay, culture and EIA, all of which are either labor intensive and time-consuming or lack sensitivity or specificity. The BD GeneOhm™ Cdiff assay (performed directly on stool specimens) offers sensitivity and specificity that is comparable to the cytotoxicity references standard and produces results in about one hour.

P1757 Comparison of an eSwab to a traditional swab for screening patients for *Staphylococcus aureus* colonisation by culture and by GeneOhm™ MRSA assay

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Objectives: *S.aureus* (SA) meticillin-resistant (MR) and-susceptible (MS), cause healthcare- and community-associated infections. The objective was to compare the performance of a Copan eSwab (a flocked swab in Amies liquid) to their conventional swab (wound swab in Stuart medium) for *S. aureus* using culture and BD GeneOhm™ MRSA (GO) (Becton & Dickinson) assay and to determine the prevalence of MRSA and MSSA in Cardiac Surgical Ward of our hospital.

Methods: Two nasal swabs were collected from 171 patients when admitted to the surgical ward. The traditional swab was processed for culture only; the eSwab was used for culture and the GO assay. All cultures were read at 24 h and 48 h. Biochemical identification and antimicrobial susceptibility testing of the isolates were performed using VITEK 2 (bioMeriéux).

Results: Of the 171 patients 35 (20.46%) had MSSA and 3 (1.75%) had MRSA isolated. All the MRSA were also identified by culture methods. In addition, culture identified 35 MSSA isolates. All the antimicrobial susceptibility testing performed on the isolates confirmed either the negative- or the positive-results of the GO assay. The eSwab samples had 1 to 2 logs of colony counts (CFU/ml) more than the traditional swabs.

Conclusion: The Copan eSwab can be used for both culture and molecular testing and provide a greater number of colonies. The GO is revealed to be a reliable and efficient assays. The combination of molecular methods with culture can improve turn around time by 24–40 hours.

P1758 Rapid detection of meticillin-resistant *Staphylococcus aureus* directly from swab culture using EVIGENE

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Background: Meticillin-resistant *Staphylococcus aureus* (MRSA) is a challenging nosocomial pathogen due to its associated morbidity, mortality, and healthcare costs. Therefore, rapid identification of MRSA carriers is essential for implementation of infection control to prevent spreading. The aim of the study was to evaluate the diagnostic

performance of parallel testing in *S. aureus* EVIGENE and mecA EVIGENE on swab cultures.

Method: 243 swabs from nares (77), throat (63), axil (30), perineum (28), inguen (11), wound (9), and other sites (25) were collected at Herlev Hospital, Denmark, and inoculated into salt-enriched semi-selective nutrient broth. After 18–24 hours incubation one aliquot was subcultured onto MRSA-ID agar and another aliquot (1.5 mL) was tested directly with both *S.aureus* EVIGENE and mecA EVIGENE.

Presumptive MRSA isolates on MRSA-ID agar plate were confirmed by colony morphology, coagulase test, cefoxitine-disc-diffusion and mecA PCR. Presumptive MRSA result found after directly testing swab cultures in EVIGENE were subcultured and isolates were re-tested in *S.aureus* EVIGENE and mecA EVIGENE as confirmation.

Results: Parallel testing in *S.aureus* EVIGENE and mecA EVIGENE showed 100% (218/218) specificity for all swab samples. Parallel testing in *S.aureus* EVIGENE and mecA EVIGENE gave no false positive result due to mixture of methicilllin-susceptible *S.aureus* (MSSA) and meticillin-resistant coagulase-negative staphylococci (MR-CNS). Sensitivity was 100% (7/7) on nasal swabs, 100% (3/3) on wound swabs, 100% (2/2) on inguen swabs, 73% (8/11) on throat swabs, and 0% (0/1) on cikatrice and 0% (0/1) mouth swabs.

Conclusion: Presumptive MRSA results from parallel EVIGENE testing (*S. aureus* EVIGENE and mecA EVIGENE) on swab cultures were available one day earlier than presumptive results obtained from MRSA-ID agar plates (24 h).

MRSA confirmation results by *S.aureus* EVIGENE and mecA EVIGENE were available one day earlier than results obtained by MRSA-ID agar plates + disc-diffusion + mecA PCR. Another, advantage of EVIGENE is that swab cultures and isolates can be tested in the same run.

P1759 Development of a electrical biochip-based detection system for rapid detection of meticillin-resistant *Staphylococcus aureus*

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Objectives: Meticillin-resistant *Staphylococcus aureus* is one of the most important pathogens causing nosocomial infections. Screening and rapid identification of patients carrying MRSA on submission to the hospital can thus significantly reduce the risk of future outbreaks and nosocomial infections caused by MRSA. The aim of our study was therefore, to develop a rapid and portable detection-system, which enables the rapid screening of patients for MRSA, and to demonstrate its use by testing clinical MRSA-isolates of epidemiological relevance, as well as challenging the detection system with clinical isolates, including coagulase-negative *Staphylococcus*-isolates. Performance of the system was compared to the PCR-based, commercially available MRSA-detection system GeneXpert.

Methods: The electrical biochip-based detection system comprises of a PCR-amplification step, targeting the SCCmec and orfX elements. Detection is achieved via a hybridisation reaction on the biochip and data are subsequently detected by generation of an electrical signal.

For the performance test, 29 MRSA-strains of epidemiological relevance were tested. Furthermore, 20 clinical isolates including coagulase-negative Staphylococci were analysed. All isolates were also checked by conventional PCR targeting mecA and nuc, and by cultivation on MRSA-Ident agar. Analysis of the strains by the GeneXpert was done according to the manufacturers instructions.

Results: With the electrical Biochip-based detection system time for analysis of MRSA could be reduced to a total of 35 min. This is significantly shorter, as compared to culture-based techniques as well as to currently commercially available rapid detection systems on the market, which take between 5 hrs to 75 min. for analysis of MRSA.

Analysis of the 29 MRSA-strains resulted in 28 correct ID's. Analysis of the 20 clinical isolates, including coagulase-negative Staphylococci resulted in 14 correct ID's. Similar results were obtained when using the GeneXpert MRSA-detection system for analysis, resulting in 29 correct ID's for the tested MRSA-strains and 14 correct ID's when analysing the clinical isolates including coagulase-negative Staphylococci.

Conclusion: These data demonstrates, that with the newly developed electrical Biochip-detection system analysis of MRSA can be achieved within only 35 min. Furthermore, the reliability of the system has been demonstrated to be comparable to the commercially available, PCR-based GeneXpert-System.

P1760 Microbiological evaluation of a new growth-based approach designated for rapid detection of meticillin-resistant *Staphylococcus aureus* directly from specimens

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Background: Active screening and compliance to appropriate infection control activities have been shown to play an important role in the control of meticillin-resistant *Staphylococcus aureus* (MRSA). Rapid diagnostic tests make these efforts even more effective. Thus, infection prevention has taken a step forward with the introduction of various tests for rapid identification of MRSA carriers. To evaluate the reliability of a new rapid screening tool, the 3M™ BacLite™ Rapid MRSA Test, to successfully detect MRSA strains, a well-characterized *S. aureus* strain collection was tested in this study.

Material and Methods: All staphylococcal strains were freshly isolated from clinical material at the University of Münster or during the course of various German multicentre studies. In total, the 724 *S. aureus* strains tested comprised 211 meticillin-susceptible *S. aureus* (MSSA) and 513 MRSA strains. To determine the clonal lineages of MRSA strains, spa typing was performed as previously described. spa clonal complexes were assigned by using the BURP algorithm. The 3M™ BacLite™ Rapid MRSA Test, that is utilising growth methodology and provides results within five hours, was used according to the instructions of the manufacturer. Ten microliters of each prepared bacterial suspension (McFarland standard 0.5) was directly transferred into each vial containing the selective broth.

Results: All 513 MRSA strains tested were recognised as MRSA, while none of the 211 MSSA strains was detected positive. These results are particularly impressive as the MRSA strains used in this study, represent more than 90 different spa types covering >90% of all registered European MRSA spa types within the SeqNet network. Beside several singletons, the MRSA enrolled in this study were grouped into eight spa clonal complexes. Thus, a very large number of MRSA strains with different genetic background were recognised as MRSA using this method.

Conclusions: The new growth based rapid MRSA assay was shown to detect without exception all MRSA strains of large collections of strains comprising highly diverse genetic backgrounds. Such a phenotypic test might be potentially more likely to cope with new strains. Further studies are warranted to evaluate this method using clinical specimens.

P1761 Specific identification of *Staphylococcus aureus* using the extracellular adherence protein gene as a novel target

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Objectives: The widely used diagnostic assays based on biochemical or immunologic reactions to identify the species *Staphylococcus aureus* present various problems including unsatisfying sensitivity, specificity, and assay time. Here, the cell surface-associated extracellular adherence protein (Eap) – mediating adherence of *S. aureus* to host extracellular matrix components and inhibiting inflammation, wound healing and angiogenesis – was analysed as target for *S. aureus* identification purposes. The feasibility of an eap-based approach was systematically analysed on genomic, transcriptional and protein levels using a large collection of *S. aureus* and non-*S. aureus* staphylococcal isolates.

Methods: A well-characterized collection of *S. aureus* (n = 597) and non-*S. aureus* staphylococcal isolates (n = 216) comprising human and

veterinary isolates from different European and non-European countries was tested for the presence of the Eap-encoding gene (eap) by PCR. In addition, Western and Northern blot analyses were performed. Clinical isolates were identified on species level using biochemical test kits. If the identification was ambiguous or categorised as unacceptable, universal target gene sequencing (16S rRNA, rpoB) was performed. PCR assays for detection of *S. aureus* and *S. intermedius* specific nuc genes were applied to confirm the identification of both species.

Results: Due to the polymorphism of the eap gene, novel PCR oligonucleotide primers for diagnostic purposes were designed. While all *S. aureus* isolates were eap-positive using the newly designed primers, this gene was not detected in non-*S. aureus* staphylococci comprising 47 different species and subspecies of coagulase-negative staphylococci and non-*aureus* coagulase positive/variable staphylococci. Furthermore, none of the non-*S. aureus* isolates expressed EAP homologs on transcriptional and protein levels. Based on these data, the sensitivity and specificity of the newly developed PCR targeting the eap gene were both 100%.

Conclusion: Eap was shown to be a virulence factor specifically restricted to *S. aureus*, occurring in all isolates of this species tested. Thus, Eap seems to be a core trait of *S. aureus*, offering a promising tool particularly suitable for molecular diagnostics of this pathogen.

P1762 Screening practices for detection of MRSA-carriers in Belgian acute care hospitals and nursing homes, 2006

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In Belgium, national guidelines for the prevention and control of MRSA are available since 1993 (updated: 2003) for hospitals and since 2005 for nursing homes (NH). In 2006, a nation-wide survey measured the implementation of these guidelines in both types of facilities.

Aims and methodology: All acute care hospitals (n = 201) and high care NHs (n = 986) in Belgium received a care type specific questionnaire, investigating the implemented measures for MRSA prevention and control. Several topics were examined: antibiotic policy, hand hygiene, isolation, additional precautions and screening and decontamination of reservoirs. This abstract reports the results of the implemented screening practices.

Results: A total of 131 hospitals (65%) and 484 NHs (49%) completed the questionnaire.

In hospitals, 95% performed “target” screening, 2% screened all patients and 4% never screened. Target screening was done after transfer from ICU (88%) and from geriatric (52%), surgical (23%) or medical wards (16%). Target screening at hospital admission was also performed for patients coming from NHs (99%), from another hospital (79%) or from a foreign country (26%). All respondents carried out nasal screening: 4% only nasal, 87% nasal combined with other sites. The combined screening of nose, throat and at least one skin site (axilla, groin or perineum) was performed by 42% of the hospitals. Rectal screening was done by 7%. Screening of the nursing staff was only carried out in epidemic situations (41%) or in specific situations: known carriers, personnel at risk, etc (13%).

Among NHs, 15% never screened residents. Screening at readmission after hospitalisation was performed by 47% of the NHs when no transfer document was available. When the transferring hospital mentioned that MRSA was isolated during hospitalisation, 66% of the NHs always screened and an additional 10% only if the resident was not decontaminated. Intermediate MRSA screening based on a combination of risk factors (3 of 4: high care dependency, presence of wound/catheter, use of antibiotics in previous month and known MRSA carriage) was done by 52%. At least 85% of the NH did nasal screening and 42% always sampled the classic ‘nose-throat-skin”.

Conclusion: Hospitals have a longer experience with screening practices and are more ‘target’ minded. In NHs, standardisation of screening practices is required. The use of transfer documents between hospitals and NHs should be optimised.

P1763 Prediction of pre-operative nasal carriage of meticillin-resistant *Staphylococcus aureus* in an outpatient evaluation clinic by using a simple questionnaire

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Objectives: To develop a questionnaire based on known risk factors for nasal carriage of Meticillin resistant *Staphylococcus aureus* (MRSA) and to test this instrument for sensitivity, specificity positive predictive value (PPV) and negative predictive value (NPV) in a preoperative outpatient evaluation clinic at the University Hospitals Leuven. This questionnaire would allow a more selective screening for MRSA carriage, the recovery rate of the systematic nasal screening for MRSA being only 1.36% at that moment.

Methods: As a standard of care, all patients presenting at the preoperative outpatient clinic are screened for nasal carriage of MRSA. A questionnaire including 7 questions (prior hospitalisation in the last year, resident of a long term care facility, antibiotic use in the last 6 months, presence of a chronic wound, presence of diabetes, healthcare worker or professional contact with animals and prior carriage of MRSA?) was developed.

During February 2007, all patients were asked to answer the questionnaire. Sensitivity, specificity, PPV, NPV, and likelihood ratio of a positive and negative test result (LR^+ and LR^-) were calculated, both when patients answered positive on 1 of the 7 questions (cut-off value of 1) and on 2 of the 7 questions (cut-off value of 2). No difference was made between the questions.

Results: 680 patients answered the questionnaire.

At a cut-off value of 1, a sensitivity of 100% and a specificity of 40% was measured (PPV 2.2% and NPV 100%, $LR^{+1.6}$ and LR^{-0}).

By using a cut-off value of 2 instead of 1, the sensitivity dropped to 67% and the specificity rose to 74% (PPV 3.3% and NPV 99%, $LR^{+2.5}$ and $LR^{-0.4}$).

Conclusion: By using a simple questionnaire with a limited number of questions and a cut-off value of 1, all MRSA positive patients were identified and a reduction of 40% of the microbiological analysises was possible. This reduction in the number of screening samples has a considerable financial impact and no consequences on the recovery rate of MRSA positive patients in our preoperative outpatient evaluation clinic.

P1764 Determination of a simple clinical score for screening of elderly patients at risk for MRSA carriage upon hospital admission in acute geriatric wards

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Objectives: MRSA transmission is an important public health problem in acute hospitals as well as in nursing homes and in the community. Furthermore, MRSA carriage plays an important role in the morbidity and mortality in the elderly. Screening at risk patients upon admission in geriatric units appears as a logical approach to prevent the spread of MRSA to frail elderly patients. We aimed to find a simple clinical score allowing to target accurately patients at higher risk of MRSA carriage while limiting at the same time both the nursing's extra-workload as well as the number of unnecessary laboratory analysis.

Patients and Methods: All consecutive patients admitted in our 27-bed acute geriatric unit from October 2006 to April 2007 were included prospectively in the trial. For each admitted patient, informations on 11 potential risk factors met in the literature were collected. Each risk factor was evaluated by an uni- and multivariate analysis (logistic regression). MRSA carriage was screened from nose, throat and skin swabs by conventional microbiological culture methods using selective chromogenic culture media.

Results: Overall 221 patients were admitted over the study period. MRSA carriage was observed in 22 patients. 6 of the 11 putative risk factors were significant by univariate analysis: age >86.9y (P = 0.002),

prior MRSA carriage ($P < 0.001$), multiple hospitalisations ($P < 0.001$), chronic wounds ($P < 0.001$), prior antibiotherapy ($P = 0.003$), long-course catheters ($P = 0.005$). Following multivariate analysis, only the first 5 criteria were retained and we computed the score for each patient (1 point per risk factor). The medians were 2/5 points (95% IC: 1.40–3.00) for the MRSA-positive group and 1/5 points (0.00–1.00) for the MRSA-negative group ($P < 0.0001$). The sensitivity, specificity and negative predictive value were respectively 72.7%, 82.4% and 96.5% (cutoff >1 point; local prevalence= 10%).

Conclusion: We propose an "easy-to-use" scoring system in order to help geriatric nursing to target and screen only the patients at higher risk of MRSA carriage upon hospital admission. Patients with a score >1 were 12.5 fold more at risk (95% IC 4.56–34.19) than those with less than 2 points. In this study, the reliance on such a clinical score would have allowed to screen only 23% of the patients. Although promising, this simple clinical MRSA scoring system should be further evaluated in a larger cohort of patients, preferably in the setting of a multicentric study.

P1765 The value of active surveillance for meticillin-resistant *Staphylococcus aureus* in orthopaedic patients

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Objectives: This project aims firstly, to identify the burden of MRSA in Orthopaedic patients and secondly, to successfully segregate MRSA patients from others who were MRSA-free

Method: This project was conducted from March to June 2007 on all Orthopaedic patients at a 1600-bedded acute tertiary care hospital in Singapore. In the first 2 weeks, all trauma and elective patients were screened for MRSA. Subsequently only high risk patients were identified for active surveillance for MRSA by the following criteria – previously known MRSA, transfers either from inter-hospital or overseas transfers, long-term care facility transfers, every 7 days amongst inpatients and end-stage renal failure patients on dialysis. Nasal swabs were taken and cultured on chromogenic MRSA agar plate after overnight incubation at the Diagnostic Bacteriology Laboratory. Besides isolation, MRSA inpatients were also given antiseptic wash to reduce bioload. MRSA outpatients listed for surgeries were given mupirocin nasal ointment, 4% chlorhexidine body wash for application prior admission, and vancomycin surgical prophylaxis. Limited isolation rooms prompted the creation of MRSA cohorts to segregate patients from those who were MRSA free.

Result: A total of 367 patients were screened, of whom 32 (8.7%) were identified as MRSA colonizers Amongst the high-risk categories, MRSA was identified in 9.3% (4/43) of trauma patients, 3.5% (6/170) elective patients, 4.5% (1/22) same-day admission patients and 11.8% (9/76) inpatients.. Of significant note is the finding of MRSA acquisition in 21.4% (12/56) inpatients who stayed longer than 7 days. Physical segregation of MRSA patients in cohort areas in open wards was difficult to achieve and maintain.

Conclusion: The study underscored the the value of active surveillance in highlighting the significant burden of MRSA in the Orthopaedic population in our setting. Hence, we have embarked on implementing in phases active surveillance as part of our patient care protocol for high-risk group patients. Detecting MRSA early may facilitate interventions that may reduce MRSA bioburden, surgical site infections, and cross transmission.

P1766 An audit of the effectiveness of measures to prevent the transmission of meticillin-resistant *Staphylococcus aureus* within a teaching hospital in the United Kingdom

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Objectives: To audit the adherence of Healthcare Workers (HCWs) within a teaching hospital to local hand hygiene policies and to evaluate patients' hand hygiene against the same audit standards.

Methods: The first audit standard was that no transmission of meticillin resistant *Staphylococcus aureus* (MRSA) should occur from a positive to a negative site on the patient's skin or their environment via HCWs' hands. The second standard was that no transmission of MRSA should occur via the patient's own hands. Three units were audited: the neuro-critical care unit, the medical intensive care unit and the department of elderly medicine. During a two-hour Observation Period for each of 24 known MRSA-positive patients (eight in each of the three locations) swabs were taken of the patient's skin, their environment, and hands of the attending HCWs. Observations were made of all hand contacts. MRSA recovered from possible episodes of transmission were typed by pulsed field gel electrophoresis, performed at the Health Protection Agency Staphylococcal Reference Laboratory.

Results: The first audit standard was met in 15 of the 24 Observation Periods (62.5%), 23 episodes of transmission occurring via HCW hands in 9 Observation Periods. The second audit standard was met in 22 (91.7%) of the 24 Observation Periods, two episodes of transmission occurring via patients' hands in two Observation Periods. For 18 of the total of 25 episodes of transmission involving either HCWs' or patients' hands, the origin of the MRSA which contaminated the hands was evident, and the patients' skin was the most common source.

Conclusion: The audit standard that transmission of MRSA should not occur via HCWs' hands was not met in a significant proportion of Observation Periods. However, the audit standard was judged unnecessarily stringent, as bacterial transmission between surfaces is difficult to avoid and prevention of transmission is unnecessary for healthcare procedures representing a negligible hazard to patients. The same principle should be applied to patients' hand hygiene. A modified approach to HCWs' hand hygiene is also proposed in the most recent international guidelines. Revising the audit standard according to these demonstrates HCW compliance in 22/24 Observation Periods (91.7%). This audit provides the first proof, based on molecular typing, that MRSA is transmitted via hands of HCW hands, and the findings also demonstrate that this is the most important mechanism of transmission.

P1767 Control of meticillin-resistant *Staphylococcus aureus* in a higly endemic setting: a 6-year experience in a teaching hospital in Slovenia

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Introduction: Endemic MRSA increases morbidity, length of hospital stay and hospital costs. In 2002, we introduced a comprehensive infection control programme (ICP) to reduce the hospital transmission of MRSA in our 230-bed tertiary care, teaching hospital in Slovenia where MRSA has become endemic.

Methods: The ICP includes use of alcohol handrub, active surveillance cultures on admission to identify MRSA carriers, barrier precautions for patients with MRSA, and eradication (decolonisation) of MRSA carriage. Selective screening was routinly performed within 72 hours of hospital admission in all patients with risk factors for MRSA carriage. Surveillance cultures performed were always obtained from the nose and throat. Additionally swabs from open wounds (when present), urine samples (when urinary catheter present >24 hours) or tracheal aspirate (intubated patients were obtained. MRSA carriers were placed in a single room, cohorted or at least 1 m between patients' beds was ensured. Gloves and gowns were required for all contacts with carriers or their

environment. Masks were required only when caring for patients with MRSA in the respiratory tract or wound.
Results: During the 6 years 42946 patients were admitted to our hospital. Growth of *S. aureus* was detected from at least 1 specimen in 2411 (0.6%) patients. Of these, 253 (10.5%) were colonised with MRSA; 70.4% were men. Two hundred fourteen MRSA cases (84.6%) were imported, and 39 (15.4%) were acquired in our hospital. In the ICU 50 MRSA carriers were detected (19.8% all MRSA carriers). Of these, 39 (78%) patients were already colonised on admission, and 11 (22%) patients acquired MRSA during the stay in our ICU. The proportion of MRSA cases acquired in our hospital declined from 35% in 2001 to 6.1% in 2002, whereas proportion of imported MRSA cases increased from 65% to 93.9%. From 2003 to 2006 the proportions of MRSA cases acquired in our hospital were 18.3%, 16.7%, 6.0, and 5.7%, respectively. Patients with imported MRSA carriage (214 cases) were transfered from other hospitals (30.4%), nursing homes (13.6%), and rehabilitation centre (0.9%) or were hospitalised at least once in the past 3 years prior to admission to our hospital (55.1%).
Conclusions: With a comprehensive ICP it is possible to reduce nosocomial transmissions of MRSA and keep them that way. A two-year increase in the number of MRSA cases acquired in our hospital was mainly due to lapses in active surveillance procedures.

P1768 Risk factors for acquisition of meticillin-resistant *Staphylococcus aureus* and clonal spread of the isolates in a medical intensive care unit

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Objectives: Meticillin-resistant *Staphylococcus aureus* (MRSA) is still commonest pathogen in hospital-acquired infections with high morbidity and mortality.MRSA colonisation usually precedes infection and dissemination of microorganism. The aim of this study was to determine risk factors for the colonisation and infection with MRSA in medical intensive care unit (MICU) and show the genetic relatedness of the strains.
Methods: This study was conducted prospectively between 1 December 2004 and 31 January 2006 in MICU. Patients (>16 years) admitted to the MICU were screened for MRSA on admission (in the first 48 hours), weekly during prolonged ICU stay, and at ICU discharge using anterior nares, axilla and groin swabs. Patients, stayed shorter than 48 hours and admitted with MRSA colonisation or infection, were not evaluated for the risk factors. Also, healthcare-workers (HCWs), close contact with these patients, were screened for nasal colonisation at the beginning and end of their ward rotation. Patient data was collected for risk factors evaluation. Genotyping analysis was performed by pulsed-field gel electrophoresis (PFGE) at the Infectious Disease Laboratory of Hacettepe University.
Results: During the study period, 259 patients were evaluated for the risk factors of MRSA acquisition. The colonisation rate was 18.5%. The mean time for MRSA acquisition was 12.7 days (range 4 to 66 days) after admission. The anterior nares were the most frequent site (87.5%) of MRSA colonisation. MRSA infection occurred in 15 (31%) of 48 colonised patients. In four patients, MRSA infection developed unless colonisation was determined. Nasal carriage of MRSA wasn't detected in HCWs during study period. In multiple logistic regression analysis, only length of stay in MICU and mechanical ventilation were the significant risk factors for colonisation. On the other hand, MRSA colonisation and tracheostomy were the significant risk factors for infection. In genotyping analysis, four clones (clone A, B, C, D) were determined in colonised patients on admission and also patients colonised in MICU had same clones (clone A, B, C). Moreover 57% of these clones were clone B.
Conclusion: This study shows the clonal spread of MRSA in an ICU and supports the results of previously published reports. Despite the efforts to improve hand hygiene (reminder posters, bedside alcohol-based products, staff education), the problem emerges because of understaffing and high workload in a developing country.

P1769 Healthcare workers' knowledge of their patients' meticillin-resistant *Staphylococcus aureus* carriage

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Objective: To assess the knowledge of healthcare workers (HCWs) knowledge of their patient's meticillin-resistant *Staphylococcus aureus* carriage in our hospital.
Method: Since 1999, a repeated prospective survey has been conducted in the teaching hospital of Bordeaux. In this 3,500 beds hospital, the infection control (IC) team daily receives a copy of the laboratory examination results when MRSA is identified. For 13 weeks per year, IC nurses systematically go to the unit where a patient is notified as carrying MRSA, they complete a questionnaire by face to face interview with the first HCW they meet regarding his/her knowledge of the carriage and record barriers precautions taken (alcohol-based handrub solution, nonsterile disposable medical gloves and gowns placed close by MRSA-positive patient). The change over the time of percentage was studied with the chi-square test for trend ($P \leqslant 0.05$ was considered to be significant).
Results: Total percentage of patients' MRSA carriage knowledge by HCWs increased significantly from 33% in 1999 to 76% in 2006. This gradual increase concerned all kind of wards. However, knowledge in the medical wards was always lower than in surgical or intensive care units (medicine wards: increase 34 to 61%, surgical wards: 29 to 81%, intensive care units 29 to 78%). Between 1999 and 2006, the frequency of barriers precautions increased gradually and concurrently with the knowledge of MRSA carriage from 74% to 94%. Furthermore, the proportion of MRSA acquired in our hospital decreased from 77% in 1999 to 62% in 2006, represented a decrease of more than 50% in nosocomial MSRA acquired during the annual study period (1.10 to 0.46 acquired SARM per 1,000 patient-days). In 2006, percentage of knowledge of MRSA carriage was higher for hospital acquired MRSA than for hospital imported MRSA (76% vs 65%; $p < 0.02$).
Conclusion: Knowledge of MRSA carriage by all HCWs is crucial for the implementation of infection control measures in hospitals, lack of information delays appropriate isolation measures. We have to improve interHCWs communication about patient carriage of MRSA. This kind of repeated active intervention is essential in infection control strategies against the spread of MRSA. Furthermore, it could help to identify units that should benefit from focused interventions to improve isolation practices.

P1770 Factors influencing the success or failure of meticillin-resistant *Staphylococcus aureus* decolonisation protocols

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Objectives: Meticillin resistant *Staphylococcus aureus* (MRSA) is a leading cause of nosocomial infection. Colonisation, often the precursor to infection, can prove difficult to eradicate with many patients remaining MRSA positive despite repeated attempts at decolonisation. This study attempts to analyse factors, which may influence the success or failure of MRSA decolonisation protocols currently in use in the Northern Health and Social Care Trust in Northern Ireland.
Methods: Restriction enzyme digestion (SmaI), followed by PFGE was used to identify strain differences in isolates from successfully and unsuccessfully decolonised patients. Successfully decolonised patients are currently being re-screened at 6 and 12 months. If positive, PFGE will be used to determine whether the patient has been persistently colonised with the same strain, or has been re-colonised with a new strain. Determination of the susceptibilities of the isolates to mupirocin was determined using E-test™.
Results: To date, 296 patients have been recruited to the study, of which 127 have been removed due to death, a worsening of their underlying medical condition or non-compliance. Sixty patients have failed decolonisation. In almost all patients examined, identical MRSA

isolates were observed before and after decolonisation. However, in a few cases, a different MRSA strain was isolated before and after decolonisation, suggesting that the patient may have been re-colonised with a second MRSA strain. Eighty-one patients were successfully decolonised, of which 31 have been re-screened at 6 or 12 months. Of these, ten patients were found to be MRSA positive, in some cases with a different strain. The susceptibilities of isolates from 42 patients, who failed decolonisation, to mupirocin were determined by E-test™. Fifteen patients (35.6%) were found to have MRSA isolates, which were considered resistant to mupirocin (MIC value >1024 mg/L). Patient medicals histories are currently also being examined

Conclusions: This study has shown that current decolonisation protocols can in many cases be successful, with a substantial proportion of patients remaining MRSA negative at 6 and 12 months post decolonisation. Failure of MRSA decolonisation protocols may be due to several patient factors (e.g. frequent contact with healthcare providers, movement between wards or failure to adequately carry out the decolonisation protocols) or due to the acquisition of mupirocin resistant MRSA.

P1771 Superspreading MRSA in a hospital ward by non-compliance with hygiene: an agent-based modelling study

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Objectives: MRSA colonisation of patients within hospital wards is largely due to indirect transmission by healthcare workers (HCWs). Here, we study transmission of MRSA in a hospital ward where HCWs may not fully comply with hygiene measures such as hand-washing. We examine whether individual non-compliance can lead to "superspreading" of MRSA.

Methods: We developed an agent-based model for MRSA transmission in a hospital ward. The model simulates patients and HCWs in a spatially explicit description of the ward. MRSA cross-transmission in patients occurs through HCWs. Two HCW profiles are allowed: one with frequent contacts with a limited number of patients (nurse profile), and the other with fewer contacts but with more patients (MD profile).

Model parameters include: epidemiological characteristics of MRSA colonisation in patients (colonisation duration of 15 days before detection and isolation or treatment) and HCWs (transient colonisation); ward organisation (mean length of stay of 10 days, HCW daily allocation and schedule); and compliance with hand hygiene. Values for the parameters are taken from the literature on MRSA and to reflect observations in hospitals.

Results: In a 20-bed ward with a 1:4 to 1:6 HCW-patient ratio, the probability of at least one secondary case of MRSA colonisation in the ward following the introduction of a single colonised patient was 52% [44%-60%] when all HCWs complied with hygiene measures. This probability increased rapidly with the presence of a few non-compliant HCWs. In the hypothesis where more than half of the HCWs were non-compliant, MRSA colonisation persisted in over 85% of cases.

When compliance decreased a little in all HCWs rather than fully in one HCW, the frequency of secondary colonisations dropped by 10%, showing the impact of heterogeneity in HCW behaviour for MRSA transmission.

In simulated epidemics, non compliant HCWs were responsible for a larger share of between patients transmission events than expected by chance. For instance, a single non-compliant HCW (representing 6% of the staff) could be responsible for 13% (for a nurse) to 21% (for a MD) of MRSA transmissions.

Conclusion: Individual non-compliance with hygiene measures among HCW can lead to epidemic outbreaks of MRSA colonisation which would otherwise have been prevented. Importantly, a global reduction in hand hygiene for all HCWs leads to smaller effects. This suggests that non-compliant HCWs may actually act as superspreaders in the hospital.

Clostridium difficile: diagnosis and treatment

P1772 Rapid detection of pathogenic *Clostridium difficile* strains and recognition of PCR ribotypes 017 and 027

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Clostridium difficile is the causative agent of antibiotic-associated diarrhoea and pseudomembranous colitis. Clinical and epidemiological data suggests that there is an increase in severity, frequency and relapse of *C. difficile*-associated disease (CDAD) in Europe and North America due to hypervirulent PCR ribotype 027 strains. Recently, outbreaks due to PCR ribotype 017 have also been noticed. Based upon the MultiFinder-technology we developed a multiplex assay to allow the identification of the new emerging 017 and 027 PCR ribotypes

Method: A set of 34 *C. difficile* strains with 9 different ribotypes was screened by Amplified Fragment Length Polymorphism (AFLP). Ribotype specific AFLP fragments were sequenced. BLAST analyses with these sequences were performed on the genome of *C. difficile* strain 630 and external primers were designed to amplify specific fragments of various PCR ribotypes. These PCR fragments were sequenced and compared with each other to identify ribotype specific polymorphisms. These polymorphisms were used for the design of ribotype specific MultiFinder probes.

Results: A set of 34 *C. difficile* strains with 9 different ribotypes was screened by AFLP. Twenty four ribotype specific AFLP fragments were isolated and sequenced. In total 43 ribotype specific polymorphisms were identified. Specific MultiFinder probes for PCR ribotype 017, 027 and a *C. difficile* universal probe were designed. In addition, probes were designed to determine the presence of toxins A, B and the binary toxin. The MLPA probes were subsequently validated on a reference set of 86 *C. difficile* strains with 22 different PCR ribotypes.

Conclusions: We developed a multiplex assay based upon the MultiFinder technology which combines the recognition of pathogenic *C. difficile* strains by the presence of toxin genes with the specific identification of PCR ribotypes 017 and 027. The specific MLPA probes for ribotype 017, 027 showed no cross reactivity with the other ribotypes. The assay, which can be performed within 6 hour, allows a fast screening without special equipment.

P1773 Development and validation of a real-time PCR assay for detection of toxigenic *Clostridium difficile*, as part of a Dutch study on the epidemiology of gastro-enteritis

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Objectives: *Clostridium difficile* is the aetiologic agent responsible for human diseases ranging from mild diarrhoea to severe pseudomembranous colitis, which are collectively referred to as *C. difficile* associated disease (CDAD). The cell cytotoxicity (CT) assay is considered the gold standard for detection of toxigenic *C. difficile*. However, this method is laborious and time-consuming (turn-around time: >48 hours). Rapid diagnosis of CDAD is important, since it may result in early treatment and help prevent nosocomial transmission. Therefore, a real-time PCR based assay was developed and validated. Also, this assay will be used as a screening tool for a gastro-enteritis study (GEops Study). This study will commence in May 2008 in 6 hospitals in The Netherlands. Its primary goal is to assess the incidence, aetiology and course of patients hospitalised for gastro-enteritis.

Methods: An assay targeting the tcdA and tcdB genes was developed. Stools were processed with the easyMAG specific A stool protocol (bioMérieux). The phocine herpes virus-1 was used as internal control. The selectivity of the assay was validated with a panel of well characterised *C. difficile* strains and clinical isolates (n = 50) and non-*C. difficile* strains (n = 43). The analytical sensitivity was assessed by dilution series (n = 3), spiked in a homogenous faecal matrix. Also, the

assay was compared with the CT assay in a clinical validation performed on stool samples (n = 163) of patients suspected of CDAD.
Results: The screening assay proved to be specific for *C. difficile* as no cross-reaction was observed. The assay was capable of detecting approximately 1560 CFU/g of stool with a 100% hit rate. Sixteen PCR positive and 139 PCR negative samples were in complete concordance with the CT assay. The PCR was positive in 8 additional samples which remained negative in the CT assay. One of these 8 samples could be confirmed as truly positive by CT in a consecutive sample of the same patient. In comparison with the gold standard (CT) PCR showed 100% sensitivity, 95% specificity, 67% positive predictive value, and 100% negative predictive value. PCR inhibition was observed in less than 1% of all 163 screened stool samples.
Conclusions:
1. This in-house real-time PCR assay offers a rapid and sensitive method for the first screening for CDAD.
2. The assay will be used as a rapid screening tool for the detection of *C. difficile* in the GEops Study that will commence in May 2008.

P1774 Development and validation of a rapid molecular screening panel for the detection of *Clostridium difficile* ribotype 027

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Objectives: *Clostridium difficile* is the aetiologic agent responsible for human diseases ranging from mild diarrhoea to severe pseudomembranous colitis, which are collectively referred to as *C. difficile* associated disease (CDAD). Since 2002, an epidemic *C. difficile* clone characterised as PCR ribotype 027, North American pulsed-field type I, and restriction-endonuclease analysis group type B is associated with higher morbidity and mortality rates, and increased antimicrobial resistance. Therefore, rapid identification methods for *C. difficile* ribotype 027 isolates are needed, since this ribotype requires modified treatment and hygienic measures. In this study real-time PCR based typing assays for *C. difficile* ribotype 027 were developed and validated.
Methods: We developed typing assays targeting the tcdC gene (delta117 and delta330–347) and the binairy toxin gene (CDTb). Stools were processed with the easyMAG specific A stool protocol (bioMérieux). The selectivity of the assays was validated with a panel of well characterised *C. difficile* strains and clinical isolates (n = 50, of which 9 were ribotype 027) and non-*C. difficile* strains (n = 43). The analytical sensitivity was assessed by a dilution series (n = 1), spiked in a homogenous faecal matrix. Also, the assays were used in a clinical validation performed on stool samples (n = 163) of patients suspected of CDAD. Samples were pre-screened for toxigenic *C. difficile* with a real-time PCR targeting the tcdA and tcdB genes. To confirm our typing assays, ribotyping PCR was performed at Leiden University Medical Centre (LUMC) on all cultured isolates.
Results: The combined results of the three typing assays proved to be specific for *C. difficile* ribotype 027, as no cross-reaction was observed. The assays were capable of detecting approximately 3×10^3, 3×10^3, and 3×10^5 CFU/g of stool with a 100% hit rate with the delta117, delta330–347, and CDTb PCR respectively. Of the 163 clinical stool samples tested, 24 were positive for *C. difficile* in the screening PCR (14.7%). By our typing assays 2 of the 24 *C. difficile* PCR positive samples were identified as *C. difficile* ribotype 027 (1.2%). Ribotyping PCR performed on cultured isolates from the same patients confirmed these 2 samples to contain ribotype 027.
Conclusion: In combination with a *C. difficile* screening PCR, these in-house real-time PCR typing assays offer a rapid and sensitive detection method for *C. difficile* ribotype 027 directly from stool specimens.

P1775 Rapid detection of hypervirulent *Clostridium difficile* strains in a diagnostic microbiological laboratory

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Objectives: The spread of hypervirulent *Clostridium difficile* strains, especially PCR ribotype 027, is a diagnostic challenge for microbiological laboratories. For rapid diagnostic purposes, we developed a simple one-tube multiplex PCR approach taking advantage of the virulence-associated markers of the hypervirulent *C. difficile* ribotype 027. The virulence profile of the *C. difficile* strains in Finnish patient samples has not been earlier determined; therefore we characterised the polymorphism in the tcdC-gene in toxin-positive *C. difficile* isolates.
Methods: A multiplex-PCR method for simultaneous amplification of *C. difficile* toxin genes (tcdA, tcdB), binary toxin genes (cdtA, cdtB) and regulator gene tcdC was developed. With this new method, we analysed consecutive *C. difficile* cultivation positive faecal samples during four weeks in November 2007. Genetic variation in tcdC gene from a representative set of the samples was analysed by sequencing.
Results: The developed multiplex-PCR method specifically identified the control strains used. From non-027 control strains toxin genes and tcdC gene were amplified. From 027 control strains, besides the toxin genes, also the binary toxin genes were amplified as well as the tcdC gene, where 18-bp deletion was clearly detectable. Of the clinical samples (n = 336), 73 (22%) were binary toxin positive and contained the 18-bp deletion in tcdC gene. TcdC gene sequencing revealed further mutations as described by Curry et al., 2007 (J Clin Microbiol 45:215–221). Two strains contained the 39-bp deletion in tcdC gene representing tcdC sequence type A as described by Spigaglia and Mastrantonio (J Clin Microbiol. 2002. 40:3470–3475). One strain with an 18-bp deletion, with a tcdC sequence corresponding to tcdC type B, and without binary toxin genes was detected. Two strains contained binary toxin genes and larger 54-bp deletion in tcdC, which has not previously been characterised to our knowledge.
Conclusion: With this method, we can easily analyse approx. 80 samples in less than six hours. The method proved specific and sensitive for identification of the *C. difficile* strains containing virulence genes associated with e.g. ribotype 027. In addition, we have initiated the development of a real-time PCR method and examination of the strains by rep-PCR. The development of rapid and reliable methods for diagnosis of these new hypervirulent strains may help to control epidemics caused by them.

P1776 Development of a real-time PCR assay for the detection of toxigenic *Clostridium difficile*

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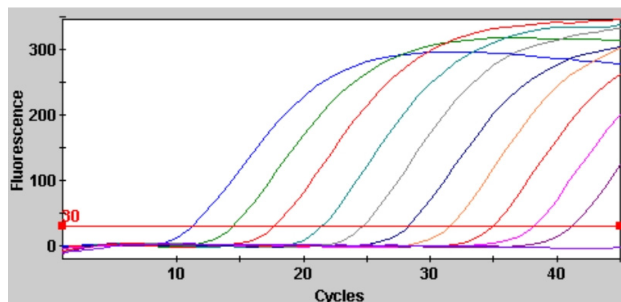
Objectives: *Clostridium difficile* (C. diff) is a common cause of nosocomial diarrhoea and incidences of infection have been on the rise in recent years. Due to increasing incidence, the emergence of a hypervirulent strain (ribotype 027), and the necessity for a sensitive, specific, and rapid test, we designed a Real Time PCR assay along with an automated extraction method for the detection of toxigenic C. diff. Typically, EIAs have a rapid turn-around-time but lack in sensitivity while the tissue culture cytotoxin assay (CTA) is sensitive and specific, but takes 24–48 hours to complete. The resultant PCR assay was retrospectively tested against clinical stool samples previously determined as positive or negative for C. diff using the Remel ProSpecT EIA. A second study (in process at the time of abstract submission) was performed prospectively on clinical stool samples comparing the PCR assay to the CTA.
Methods: The C. Diff molecular assay utilised the bioMérieux Nuclisens easyMAG for automated nucleic acid extraction. Real Time PCR using Taqman chemistry was performed on the Cepheid SmartCyclerII. Specificity was assessed by testing a variety of organisms that present the same symptoms as C. diff. The retrospective study analysed 363 stool samples (176 positive and 187 negative by the EIA). The retrospective and prospective studies were used to evaluate the clinical performance of the PCR assay.
Results: The PCR assay detected the Positive Control down to 5 copies per reaction (Figure 1). The PCR assay did not cross-react with any of the specificity organisms tested. Retrospective testing resulted in 164 PCR positive of 176 EIA positive samples and 173 PCR negative of 187 EIA negative samples. Two of the EIA negative samples were inhibited resulting in an inhibition rate of 0.55%. Twelve of the EIA negative

samples were positive by PCR and 12 of the EIA positive samples were negative by PCR. These results led us to perform prospective testing in comparison to the CTA as false positives and negatives have been reported when using EIAs for C. diff detection.



Dynamic range of the PCR assay. Positive Control DNA was serially diluted from 5×10^9 to 5×10^{-1} copies/reaction.

Conclusion: We developed a Real Time PCR assay that is sensitive, specific, and can be performed in less than 4 hours. With the increased incidence of C. diff associated disease and the emergence of a hypervirulent strain, it is imperative that a reliable, accurate, and rapid test is available to clinical laboratories to aid in diagnosis and treatment of C. diff.

P1777 Relapsing *Clostridium difficile*-associated diarrhoea: predictive value of faecal toxin assay and anti-*Clostridium difficile* whey protein to aid prevention of relapses

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Background: Antibiotic treatment generally is effective to attain symptomatic recovery in *Clostridium difficile*-associated diarrhoea (CDAD), but many patients suffer relapses of diarrhoea. An sIgA-enriched immune whey (Anti-CD-WPC) made of milk of immunised cows may reduce CDAD relapse rate; in those who relapse still, the dose of whey may be insufficient to neutralize completely faecal toxins in CDAD. Herein, we assess the ability of the standard dose of Anti-CD-WPC to neutralize faecal toxins, in relation to relapses of CDAD.
Methods, design, patients: Prospective cohort study of Anti-CD-WPC-40% in 107 epsiodes in 100 consecutive patients with CDAD (cf. Gut 2007;56(6):888). After completion of 10 days of Metronidazole (n = 48), Vancomycin (n = 56) or both (n = 3), patients received Anti-CD-WPC-40% 5 g tid for two weeks (Day 1–14), with a follow-up period of 60 days. Outcome measures were the occurrence of relapse CDAD, faeces *C. difficile*-toxin (Vidas, BioMerieux) and culture positivity during (on relapse) and 4 days after completion of Anti-CD-WPC (Day 18).
Results: Eleven of 107 CDAD episodes (~10%) were followed by a relapse. Seven patients relapsed before day 18: all tested toxin and culture positive at relapse, despite use of (n = 4) or shortly after (n = 2) the course of Anti-CD-WPC. An additional 4 patients relapsed between Day 21 and 40. On Day 18, 4 days after completing Anti-CD-WPC, a positive faeces toxin assay and/or positive *C. difficile* culture were strongly associated with risk of subsequent relapse (RR 8.20, 95%-CI 1.04–64; and RR 4.7, 95%-CI 0.5–47, respectively). Moreover, in 18 patients toxin assays were performed on Day 5–7 while taking Anti-CD-WPC: one of 4 toxin positive patients relapsed during Anti-CD-WPC, as compared to none of 14 cases assayed negative.
In an additional 5 CDAD cases, toxin assays were positive during Anti-CD-WPC-40% but reverted to negative upon doubling the Anti-CD-WPC dose.
Conclusion: Patients at high risk for CDAD relapse despite taking immune whey are those still testing positive for faeces toxins while taking or shortly after stopping Anti-CD-WPC. In these, Anti-CD-WPC-40% 5 g tid apparently is insufficient to fully neutralize *C. difficile* toxins. It should now be investigated whether individually titrating on the basis of the faeces toxin test the amount (e.g., 5 g Anti-CD-WPC-80% instead of -40%) or extend the use of Anti-CD-WPC >14 days can increase further its efficacy to help prevent CDAD relapses.

P1778 Antimicrobial susceptibility patterns of *Clostridium difficile* from two tertiary referral trusts within Birmingham, UK

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Objectives: To determine and compare the antibiogram profiles of sixty two clinical isolates of *Clostridium difficile* recovered from patients within two tertiary referral Trusts, Birmingham, UK. Antibiogram profiles of *C. difficile* NCTC 11204 and *C. difficile* Ribotype 027 (Anaerobic Reference Laboratory, Cardiff) were also determined.
Methods: Minimum Inhibitory Concentrations (MIC) were determined by agar dilution. Antibiotics tested were: cefotaxime, chloramphenicol, clindamycin, erythromycin, fusidic acid, imipenem, levofloxacin, linezolid, metronidazole, rifampicin, tetracycline and vancomycin. Twenty four hour cultures of *C. difficile* grown in Wilkins Chalgren Anaerobe Broth were adjusted to 10^7 CFU/ml and 10 microlitres inoculated onto the surface of the antimicrobial Wilkins Chalgren Anaerobe Agar. Inoculated plates were incubated anaerobically for 48 hours at 37ºC. MICs were determined as the lowest concentration where growth was inhibited. Breakpoints used were pharmalogical non species specific breakpoints defined by the Swedish Reference Group for Antibiotics unless European Committee on Antimicrobial Susceptibility Testing breakpoints were available.
Results: Varying degrees of resistance was observed against all antibiotics tested with the exception of vancomycin and metronidazole. Resistance towards fusidic acid and cefotaxime occurred in 92% and 98% of isolates respectively. Levofloxacin resistance was seen in 89% of isolates and clindamycin, tetracycline and erythromycin resistance was 78%; 73% and 70% respectively. Resistance among isolates was less common towards rifampicin (53%), imipenem (41%), chloramphenicol (20%) and linezolid (2%). Of all the isolates tested only 40% were sensitive to $\geqslant$6 of the antibiotics. Ninety-three percent of isolates recovered from one Trust were resistant to $\geqslant$6 of the antibiotics, compared to those obtained from the second Trust where only 34% exhibited resistance to $\geqslant$6 of the antibiotics.
Conclusion: Whilst all isolates of *C. difficile* in this study retained sensitivity to vancomycin and metronidazole, resistance to other antibiotics is widespread. Resistance patterns are likely to be governed by location and antibiotic prescribing policy within individual Trusts. Furthermore the antibiogram profiles obtained highlight phenotypic differences which may be used potentially to differentiate between strains and indicate differences at the genetic level.

P1779 An investigation into antibiotic susceptibility and ribotype profiles among community-acquired and hospital-acquired *Clostridium difficile* strains isolated in a university teaching hospital laboratory

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Clostridium difficile associated diarrhoea (CDAD) is associated with many risk factors, the most significant being previous antibiotic exposure. We currently diagnose *C. difficile* by toxin detection. Culture, antibiotic susceptibility testing and ribotyping are reserved for outbreaks or therapeutic failures.
Objectives: The aim of this study was to determine the susceptibility of toxin positive *C. difficile* isolates to Metronidazole, Vancomycin, Ciprofloxacin and Cefotaxime, to correlate this with previous antibiotic exposure, and to compare antimicrobial sensitivities and ribotypes in isolates from hospital and community acquired (CA) cases.
Methods: Over a period of 6 months, 200 toxin positive stool samples, including 24 CA samples, submitted to a large hospital laboratory in Glasgow were cultured for *C. difficile*. All isolates were tested for antimicrobial susceptibility using E-strips (AB Biodisk). PCR-ribotyping was carried out on a subset of isolates comprising all CA cases and

time matched hospital cases. Community acquired cases were defined as showing symptoms within 48 hrs of admission with no history of hospitalisation in the previous 3 months. Antibiotic history was obtained from patient records.
Results: *C. difficile* was isolated from 184 stool samples. All isolates were sensitive to Metronidazole and Vancomycin. Resistance to Ciprofloxacin was detected in 179 isolates (97.3%) and to Cefotaxime in 173 isolates (94.0%). Four isolates (2.2%) were sensitive to all antibiotics tested.
Ribotyping results were obtained for 45 isolates. The most common ribotypes were 106 (29%), 001 (26%), 005 (7%), 014 (7%), and 017 (4%). Most ribotypes were obtained from both hospital and CA cases. Ribotypes 002 and 027 were only identified among CA cases, while 078 was identified among hospital cases.
The ribotype patterns obtained for 6 isolates have yet to be matched with patterns from the UK database.
Conclusion: There was no correlation between antibiotic susceptibility and previous antibiotic exposure. The data are still being assessed for ribotype correlation with antimicrobial susceptibility patterns.
Recent data from Scotland (Mutlu et al, 2007) suggested ribotype 001 was approximately 10 times more common than ribotype 106, however in the current study this was not the case, and the distribution of ribotypes 001 (26% overall) and 106 (29% overall) are quite similar.
This is the first time ribotype 027 has been isolated from the community in Scotland.

P1780 Antimicrobial susceptibility and molecular epidemiology of *Clostridium difficile* (CD) isolated from faecal CD toxin-positive patients in a UK teaching hospital

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Objectives: *Clostridium difficile* causes a range of important nosocomial infections varying from diarrhoea to pseudomembranous colitis, known collectively as *C. difficile*-associated disease (CDAD). In Nottingham University Hospitals NHS Trust there has been an increase in severe cases of CDAD, which, in turn, has increased morbidity and mortality. At present CDAD is diagnosed using enzyme immunoassay, but this does not provide any information on antibiotic susceptibility or epidemiology. This study aimed to determine the local epidemiology and antimicrobial susceptibility of *C. difficile* in Nottingham.
Methods: Between 1 April and 8 June 2006, 100 *C. difficile* toxin-positive faecal samples were cultured using the alcohol shock method. Susceptibilities to metronidazole, vancomycin, cefuroxime, moxifloxacin and erythromycin were determined using a modified Stoke's method, and the isolates were typed using 16–23S rRNA PCR-ribotyping. Isolates that were non-UK epidemic ribotypes were examined by PCR for the toxin A and toxin B genes; isolates that were toxin A-negative were further examined for deletions in the toxin A gene.
Results: All 100 isolates were metronidazole-sensitive, vancomycin-sensitive and cefuroxime-resistant. Most (n = 86) isolates were moxifloxacin-resistant and 82 isolates were erythromycin-resistant. In total, 61 isolates belonged to ribotype 106, 18 to ribotype 027, seven to ribotype 001, and 14 to other non-UK epidemic ribotypes. The majority of the non-UK epidemic ribotypes (11/14) were toxin A-positive and all were toxin B-positive. PCR amplification of the toxin A gene (tcdA) demonstrated two different deletions in the 3′-end of the tcdA gene.
Conclusion: The predominant local epidemic strain of *C. difficile* belonged to ribotype 106. Ciprofloxacin, erythromycin and cefuroxime are commonly prescribed antibiotics in Nottingham, and this antibiotic policy is likely to be a contributing factor to the selection of antibiotic-resistant *C. difficile* ribotypes in Nottingham. There is evidence that the tcdA-negative isolates detected in this study may have novel deletions in the tcdA gene, and that these isolates may play a role in the evolving local epidemiology of CDAD.

P1781 Characterisation of moxifloxacin-resistant *Clostridium difficile* mutants selected in vitro

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Objectives: To perform an analysis of point mutations in the quinolone resistance-determining region (QRDR) of the DNA gyrase of *Clostridium difficile* strains after "in vitro" exposure to increasing concentrations of moxifloxacin (MX).
Methods: Five MX susceptible *C. difficile* strains were selected: strain C253 and 630 (toxinotype 0), strain CD5 (toxinotype V), strain A422 and BI1 (toxinotype III). MIC values for MX were determined using the Agar Dilution method. The breakpoint used was 8 mg/L. Selection of MX-resistant mutants was performed by growing bacteria on Mueller-Hinton (MH) plates with twofold increases in concentrations of MX (from 2 to 32 mg/L) for 48 h. Resistant mutants were grown for three repeated passages on MH plates without MX. The QRDRs of gyrA and gyrB were amplified and sequenced.
Results: Before induction, all *C. difficile* strains had MICs for MX between 1 and 2 mg/L. No strain showed mutations in GyrA or GyrB, except CD5 that had a mutation in GyrB already described in susceptible strains. BI1 resistant mutants, isolated from the first step of selection (2 mg/L of MX), showed the substitution Arg447 to Lys in GyrB. At the fourth step (16 mg/L of MX) these mutants acquired another mutation (Asp426 to Asn) in GyrB. MICs of BI1 mutants were comprised between 16 and $\geqslant$32 mg/L. C253, 630 and A422 mutants were isolated from the third step of selection (8 mg/L of MX) and showed the substitution Ala118 to Ser in GyrA, Asp426 to Val in GyrB and Asp81 to Asn in GyrA, respectively. The third mutation resulted new. MICs of C253 mutants ranged 8–16 mg/L, those of 630 mutants were 16 mg/L, whereas MICs of A422 mutants ranged 16–24 mg/L. Two different CD5 mutants were isolated: those from the second step (4 mg/L of MX) showed the new mutation Ala92 to Glu in GyrA, whereas those from the third step (8 mg/L of MX) showed both mutations Ala118 to Ser and Thr82 to Ile in GyrA. The MICs of the mutants with Ala92 to Glu ranged 4–8 mg/L, whereas those of the mutants with Ala118 to Ser and Thr82 to Ile were comprised between 16 and $\geqslant$32 mg/L.
Conclusion: These results demonstrate that MX is able in vitro to generate different mutations in both GyrA and B of *C. difficile* strains. One or two mutations can be present in the same protein and two new mutations were identified in GyrA. The apparent easiness in developing resistant mutants may explain the circulation of highly resistant strains, such as *C. difficile* ribotype 027, in the hospital environment.

P1782 Susceptibility of *Clostridium difficile* spores to copper surfaces: the Germination Theory

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Objectives: Over the past decade the number of cases of *Clostridium difficile* associated disease has risen significantly and *C. difficile* is now the leading cause of nosocomial diarrhoea in the UK. Contributing factors include the persistence of *C. difficile* spores on surfaces in the clinical environment, and their resistance to common antimicrobials. However, it is well recognised that germinating bacterial spores demonstrate increased susceptibility to many antimicrobials. The antimicrobial activity of copper surfaces against many microorganisms is well documented; however there has been no research into the efficacy of copper against spores of *C. difficile*. The aim of this investigation was to determine the antimicrobial activity of copper surfaces on dormant and germinating spores of *C. difficile*.
Methods: A controlled carrier test system was designed comprising inoculation of copper and control stainless steel discs with ten microliters of a 10^6 CFU/ml spore suspension of *C. difficile* NCTC 11204, in aerobic conditions to simulate the clinical environment. The antimicrobial efficacy of copper surfaces against dormant spores of *C. difficile* and spores exposed to the germinant 1% (w/v) sodium taurocholate was assessed over a 3 hour period, with sampling every 30 minutes. Each

sample was neutralised with D/E neutralising broth before culturing onto Fastidious Anaerobe Agar (containing 0.1% sodium taurocholate).
Results: There was no log reduction in the number of *C. difficile* spores recovered from control stainless steel discs, both in the presence and absence of sodium taurocholate over 3 hours. When spores of *C. difficile* NCTC 11204 were exposed to copper, without the incorporation of sodium taurocholate, there was no log reduction. However, in the presence of the germinant, there was a 3.54 log reduction (99.9% reduction) in the number *C. difficile* spores after 3 hours.
Conclusion: The antimicrobial efficacy of copper surfaces against germinating spores of *C. difficile* NCTC 11204 is clearly demonstrated in this study. Incorporation of copper surfaces and the use of a germinant solution within the clinical setting may facilitate the reduction of infection due to *C. difficile*. Further investigation is warranted.

P1783 Efficacy of faecal therapy for recurrent *Clostridium difficile*-associated diarrhoea

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Objectives: Recurrent *Clostridium difficile* associated diarrhoea (CDAD) is an increasing problem partly due to the highly virulent ribotype 027 strain. Antibiotic therapy for a first recurrence of CDAD fails in approximately 55% of cases. Treatment of subsequent recurrences is even less successful illustrating an urgent need for more powerful treatment strategies. We describe our results with faecal therapy in patients with recurrent CDAD.
Methods: Patients with a proven relapse of CDAD (diarrhoea and positive ELISA for *C. difficile* toxin) after at least 2 courses of antibiotic treatment were included. Patients were pre-treated with vancomycin 500 mg qid during 4–7 days followed by bowel lavage with polyethylene glycol and infusion of donor faeces in the cecum or jejunum. Fresh donor faeces were donated by healthy relatives of the patients who were screened for infectious agents (intestinal pathogens and parasites, HIV, and hepatitis viruses). Donor faeces (150–200 g) were dissolved into a 400 cc solution with 0.9% saline. After infusion of donor faeces resolution of diarrhoea and clearance of faecal *C. difficile* toxin was assessed.
Results: Eight patients (aged 48–82 years, 5 males and 3 females) received faecal therapy. Two patients were infected with the ribotype 027 strain. Patients had been treated with antibiotics for CDAD during a median time of 70 (range 55–139) days prior to faecal therapy, and isolation precautions were required during a median duration of 80 (range 72–151) days for hospitalised patients (n = 6). In 6 of 8 patients, diarrhoea resolved within 4 days after donor faeces infusion. In 2 patients, CDAD recurred but a succesful outcome was achieved with subsequent courses of faecal therapy using another stool donor. *C. difficile* toxin test and culture were repeatedly negative after resolution of diarrhoea in 7 of 8 patients. One treated patient was considered an asymptomatic carrier with a persistant positive toxin test and culture, with absence of diarrhoea. One patient died 4 weeks after faecal therapy due to respiratory failure unrelated to donor stool infusion. One patient had another episode of CDAD, four months after faecal therapy.
Conclusion: Faecal therapy seems a promising treatment strategy for recurrent CDAD, also in patients infected with the hypervirulent ribotype 027 strain. A randomised trial to compare the efficacy of faecal therapy with conventional antibiotics for recurrent CDAD has been initiated.

P1784 In vitro selection of probiotic multispecies against *Clostridium difficile* 027

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Clostridium difficile (CD) ribotype 027 is an emerging disease causing severe outbreaks of diarrhoea in hospitals as wel as the community in Europe, North America, Canada and Japan. Treatment options for *C. difficile* associated diarrhoea (CDAD) consists of preventive and treatment measures i.e. controlled use of antibiotics, hygiene measures and treatment with vancomycin or metronidazol. Still mortality (>10%) and mutiple relapses (>20%) are persisting challenges to overcome. Sofar probiotics have no place in the treatment or prevention of 027 associated CDAD due tot lack of convincing clinical studies and severe side effects e.g. the yeast *Saccharomyces boulardii*. Most of the clinical studies are carried out with only one or a limited numer of probiotic strains. Still many lactic acid producing probiotic species like lactobacilli, bifidobacteria and lactococci have found to inhibit CD. We studied the inhibitory potential of 28 probiotic species against 6 CD 027 and non-027 strains in solid as well as liquid culture. Whereas all CD strains were equally effected, substantial differences were found between probiotic strains even within one specie. Probiotic whole cell cultures (cells + extracellular components) were found to be significantly more effective than extracellular components only. Remarkably no correlation was found between CD growth inhibition and pH nor the natural production of lactic acid. Probiotic multispecies were found more effective against CD 027 in liquid co-cultures as compared tot singlespecies. Still single probiotic species were found to inhibit toxin production despite a failure in reducing growth of CD 027. Co-culture in solid as well as liquid media may be very helpfull in selecting potential combinations of probiotic multispecies for clinical treatment or preventive studies against *C. difficile*.

P1785 Vaccinal strategy against *Clostridium difficile* associated disease with surface proteins of *C. difficile*

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Clostridium difficile is a cause of enteric diseases ranging from mild diarrhoea to severe pseudomembranous colitis, particularly after antibiotic treatment. The two toxins A and B are the main virulence factors. In addition, a number of bacterial virulence factors associated with adherence to the gut are implicated in the first step of pathogenesis, the colonisation process. Blocking the primary stages of infection, namely bacterial attachment to host cells and colonisation of the mucosal surface, may be an effective strategy to prevent *C. difficile* infection.
Objectives: In this study, using the hamster model of *C. difficile* infection, we assessed the immunogenic and protective effect of cell wall extract of a non toxigenic strain of *C. difficile* and the protease Cwp84 used as vaccine antigens for mucosal immunisation.
Methods: Hamsters were divided in three groups immunised respectively with: PBS for the control group, the recombinant protease Cwp84 for the second and cell wall extract of a non toxigenic strain of *C. difficile* for the last group.
After three immunisations by the rectal route with antigens combined with Cholera toxin as adjuvant, hamsters received Clindamycine. Then, five days later, they were challenged by a toxigenic stain of *C. difficile*. Intestinal colonisation and post-challenge survival was followed. For all groups, animal sera were sampled before and after immunisation for immunological analysis. Sera of hamsters immunised with the protease Cwp84 were analysed by ELISA and sera of hamsters immunised with the cell wall extract were analysed by two-dimensional electrophoresis coupled with an immunoblot and mass spectrometry in order to reveal the most immunogenic proteins.
Results: In the two immunised groups, survival was prolonged as compared to the control group with a statistically significant difference. Enumeration of *C. difficile* in faecal samples showed that survival animals were not colonised by *C. difficile*. As the immune response analysis is concern, the specific antibody level observed by ELISA between the control group and the group immunised by Cwp84 was not significantly different. For the group immunised by cell wall extract, the most immunogenic proteins observed by immunoblot in the animal sera were identified.
Conclusion: These results suggest that mucosal immunisation with surface proteins could at least partially protect the hamster against *C. difficile* infection.

P1786 **The dilemma of reporting culture-positive, toxin-negative *Clostridium difficile* results on patients with diarrhoeal stools**

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Objectives: *C. difficile* (CD) is a major cause of hospital-acquired diarrhoea. Production of TcdA and/or TcdB toxins is the primary virulence factor, although the binary toxin CdtB may also be important. Because strains in our institution are used for epidemiological and susceptibility studies, both culture and toxin detection are performed. The purpose of this work was to investigate if tests where toxin was negative but culture was positive represented *C. difficile* associated disease (CDAD) and should be reported as CD positive.

Methods: From strains isolated from specimens submitted for CD testing from patients with diarrhoeal stools, we selected only those strains originally culture positive and toxin negative. These CD strains were first screened after subculture to detect TcdA and/or TcdB employing the ELISA test directly from the colony. If negative, PCR for the detection of tcdB was performed. Further analysis consisted of detecting cdtB and typing by pulse field gel electrophoresis (PFGE).

Results: During the 2005–2006 period 1891 specimens were processed, 67% were culture–/toxin–, 16% were culture+/toxin+, 6% were culture–/toxin+ and 11% were culture+/toxin–. Of the 215 strains that were culture+/toxin–, we assumed that the 135 strains that were positive for toxin on a prior or subsequent specimen from the same patient would likely be toxin positive (based on previous work) and were not further analysed. Of the rest, 68 strains were available for further testing and 40 of these tested toxin positive from the colonies. Of the 28 strains that were further tested 8 had the tcdB gene (7 were toxinotype O and one was toxinotype VIII). None of the 20 strains that were tcdB negative carried the cdtB gene and their PFGE type did not resemble other known strains. The clinical history of these 20 patients indicated that none had been treated for CDAD. The clinical history of patients for whom we had reported CD culture+/toxin– but whose strains were eventually shown to produce toxin showed more disease and treatment.

Conclusion: Detection of toxin from the colony is helpful but not as sensitive as molecular methods. Most of the initial culture+/toxin– reports represented strains that were eventually shown to be toxin producers. The culture+/toxin– report was important in that it sometimes predicted a subsequent toxin positive report or was the only positive result in a patient who had CDAD.

Candidaemia and other *Candida* infections

P1787 **Candidaemia in a tertiary hospital: analysis of trends and mortality**

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Background: Candidaemia is associated with significant mortality and morbidity.

Methods: Retrospective review of the episodes of candidaemia diagnosed from Jan.00 to Dec.05 in our Centre. We include all episodes with, at least, 1 positive BC yielding *Candida* sp. Clinical, microbiological and epidemiological data were obtained and analysed. Candidaemia was considered nosocomially-acquired (N-A) if the diagnosis was done $\geqslant$72 h after hospital admission (HA) also pts diagnosed after discharge within 60 d of a previous admission.

Results: 146 episodes of candidaemia were identified. Mean age was 54 yrs-o (2 m-96 yrs-o). Mean time from HA to candidaemia was 27 d (0–150, ±25). 89% were considered N-A. Origin of candidaemia was catheter-related (CR) in 40%, followed by primary candidaemia(PC) (30%), urinary tract infection (UTI) (10%) and intrabdominal infection (IAI) (9%). 51% were *C. albicans* (CA) and 48% were caused by non-*albicans* species (NA-C). 39% were oncohaematologic (OH) patients. In OH-pts is more frequent N-A spp. (55%). 11% have neutropenia. Attending to the site of admission:medical wards (42%), ICU (39%), surgical wards (19%). In CR infection the most frequent isolated was *C. parapsilosis* (57.6%). *C. albicans* was the predominant species in PC (53.5%), UTI (64%) and IAI (61%). N of episodes of candidaemia per year has increased (15 cases/yr in 2000 to 32 cases/yr in 2005). The distribution of CA and NA-C spp. per year remains stable. Mortality during 1st month was 39%. Mean time from candidaemia to exitus was 11 d (0–30, ±9.1). Mortality rates varies according to the different origin of candidaemia: CR (32%), PC (44%), UTI (28.6%), IAI (23%).

In univariate analysis of mortality, N-A ($p=0.001$), age $\geqslant$50 yrs-old ($p=0.02$), presence of metastatic neoplasia ($p=0.004$), shock ($p<0.001$), renal failure ($p=0.005$), respiratory failure ($p=0.01$) and receiving antifungal therapy <5 d ($p<0.001$) were associated with mortality.

Conclusions: The number of cases of candidaemia per year is increasing. The distribution of CA and NA-C spp. remains stable. The most frequent source of candidaemia was catheter-related. Candidaemia has a high mortality rate. Age, presence of metastatic neoplasia, shock, renal failure, respiratory failure and receiving antifungal therapy <5 d was associated with mortality in univariate analysis.

P1788 **Incidence of candidaemia: 5-year results from emerging infections in patients from the intensive care unit of a tertiary hospital**

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Introduction: The incidence of severe *Candida* bloodstream infections (BSI) is rapidly increasing since the 90‘s and it is becoming a major cause of morbidity and mortality in intensive care unit patients. *Candida* species are now the fourth most common cause of hospital-acquired bloodstream infections. Although the incidence of candidaemia among previously hospitalised patients increased, recent reports suggest that the incidence has stabilised.

Objectives: The purpose of this study was to know the incidence and the tendency of candidaemia during 5 years in an Intensive Care Unit (ICU) from a tertiary hospital.

Methods: A case of candidaemia was defined as the incident isolation of *Candida* spp. from a blood culture. Candidaemia occurring >30 days after the incident isolation was defined as a new case. To calculate incidence rates, the numbers of admissions and patient-days were collected. Incidence rates were calculated as the number of candidaemias per 1,000 admissions. To compare the tendency of annuals incidences of candidaemias Chi-square test was performed.

Results: We collected a total of 77 consecutive episodes of *Candida* BSI in adults from ICU. *Candida albicans* was the most prevalent isolated species ($n=35$); the remaining 42 strains were *Candida parapsilosis* ($n=29$); *Candida glabrata* ($n=4$), *Candida tropicalis* ($n=2$); *Candida krusei* ($n=2$); *Candida guilliermondii* ($n=1$) and other *Candida* spp. ($n=4$). The incidence of BSI of *Candida albicans* rate per 1000 admissions and by year were 2.39, 2.71, 2.87, 2.62 and 3.59; and other *Candida* non-*albicans* 2.39, 4.51, 3.28, 3.37 and 3.59. The overall incidence of BSI of *Candida* rate per 1000 admissions was 7.07, (4.78, 7.22, 6.16, 5.99; 7.18). No significative diferences were found among the annual incidence rates of candidaemia in ICU patients: $p=0.8344$ (whole candidaemias); $p=0.9453$ (*Candida albicans*) and $p=0.835$ (non-*albicans Candida*).

Conclusions: Although the study period was reduced, the results do not reveal significative changes in the incidence rates of all candidaemia episodes caused by *Candida albicans* and non-*albicans Candida*.

P1789 **Risk factors associated to mortality in intensive care unit patients with candidaemia**

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Introduction: After technological and scientific advances during the last three decades, invasive fungal infections turned to emerging infections associated to important morbidity and mortality rates. Medical and surgical ICUs have become the epicentre of candidaemia.
Objectives: Identify main risk factors associated to mortality in intensive care unit (ICU) patients with candidaemia in the Complejo Hospitalario Universitario de Santiago de Compostela in Galicia, Spain.
Methods: We carried out a retrospective study of candidaemia episodes collected at the Complejo Hospitalario Universitario de Santiago de Compostela Microbiology Laboratory from January 2000 to December 2005. We selected the candidaemia isolations from the medical and surgical critical care units. The association study between qualitative variables has been carried out by means of the chi-square test and the Fisher exact test. In order to identify predictive variables for mortality, univariate and multivariate logistic regression models have been adjusted. The following independent variables were analysed: sex, abdominal surgery in the last three months, parenteral nutrition, antifungal treatment, antibiotic treatment, inmunosupresor treatment, catheterisation, mechanical ventilation, *Candida albicans* fungaemia and being more than 65 years old.
Results: During the study, 77 episodes of ICU-acquired candidaemia were identified. Mortality rate was 50%, but it was higher in patients with *Candida albicans* fungaemia (odds ratio [OR], 3.1; confidence interval of 95% [CI], 1.2 to 7.9; $P < 0.05$), in patients older than 65 years ([OR], 4.4; 95% [CI], 1.6 to 11.7; $P < 0.05$) and in women ([OR], 2.9; 95% [CI], 1.1 to 7.6; $P < 0.05$). On the contrary, no significant associations between mortality and the rest of the analysed variables were observed.
Conclusion: Candidaemia was associated with a high mortality rate in our ICU. The main risk factors associated with mortality were *Candida albicans* fungaemia, advanced age and female sex.

P1790 **Trends in incidence of candidaemia in ICU: an eight-year experience**

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Objective: *Candida* spp. is the most important non-bacterial pathogen in critically ill patients (pts). The aim of this study is to evaluate trends in incidence of candidaemia and distribution of *C. albicans* and non-*albicans* over a 8 years period (1999–2007) and to assess its relationship with fluconazole (fluco) use.
Methods: Cases of candidaemia were retrospectively screened. Fluco use (in defined daily doses, DDD) and incidence of candidaemia are expressed per 10000 patients/day for each year of evaluation.
Results: During the 96-month-period a total of 213 episodes of candidaemia (average incidence 1.42 episodes/10000 pts-day/year, range 0.36–3.02 episodes) were collected in a mixed medical and surgical ICU in S. Martino Hospital in Italy. *C. albicans* was the most prevalent isolated species (n = 98, 46%); the remaining strains (n = 115, 54%) were non-*albicans*, more represented by *C. parapsilosis* (n = 40, 35%) and less by *C. glabrata* (n= 28, 25%). The incidence of candidaemia by year and species and fluco consumption are indicated in the table 1.

Table 1

	1999	2000	2001	2002	2003	2004	2005	2006	2007
Candidaemia/10,000 pts-day	0.84	1.53	1.79	2.62	3.02	0.36	0.39	0.44	0.37
Candidaemia due to *C. albicans* /10,000 pts-day	0.52	1.2	0.6	0.7	0.73	0.15	0.34	0.33	0.37
DDD of fluco /10,000 pts/day	36	44	57	108	86	74	57	63	38

Conclusion: The study showed that the incidence of candidaemia decreased in the last 4 years of observation. Incidence of candidaemia due to non-*albicans* species increased until 2003 and this was apparently correlated with an increasing use of fluco for prophylaxis. *C. albicans* candidaemia remained stable during the eight years. The knowledge of the local epidemiological trends in *Candida* spp. isolated in blood cultures is important to guide therapeutic choices.

P1791 **Candidaemia: incidence, risk factors, characteristics, and outcomes in immunocompetent critically ill patients**

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Objective: To determine the risk factors for development of candidaemia in patients requiring intensive care unit (ICU) treatment for more than 48 h and to study the role of antifungal prophylaxis in the development and outcome of candidaemia.
Methods: A matched case control study was conducted. Patients admitted to the medical-surgical ICU who developed candidaemia after at least 48 h were matched to a control group of ICU patients according to five variables: length of ICU stay until development of candidaemia, age, admission department (medical, surgical and ICU), year of admission and sex.
Results: Forty-five patients with candidaemia were enrolled in the study; 46 yeasts were isolated from blood cultures. The incidence of candidaemia was 0.6 cases per 1000 patient days. *Candida albicans* was the most commonly isolated yeast followed by *C. tropicalis*. The risk factors associated with development of candidaemia were *Candida* colonisation, duration of mechanical ventilation, GIT surgery, total parenteral nutrition, absolute lymphocyte count <1000/μl at the day of the positive blood culture, and diabetes mellitus. In the regression analysis *Candida* colonisation and GIT surgery were independently associated with candidaemia development. Prophylactic administration of antifungal agents was not associated with better outcomes or non-*albicans* candidaemia. ICU and total in-hospital mortality rates were 40% and 66.7%, respectively. Candidaemia related mortality was 20%. Candidaemia treatment failure was the only variable associated with in-hospital mortality ($p = 0.008$).
Conclusions: Candidaemia mainly develops in critically ill patients with multiple organ failure and end-stage disease and it is associated with increased morbidity and mortality. Patient populations that may benefit from preventive measures, including the use of antifungal prophylaxis, should be further studied.

P1792 **Survey of candidaemia in a tertiary care hospital in Greece.**

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Objectives: *Candida* is an important pathogen in the hospital setting. The aim of this study was to analyse the morbidity and mortality related to candidaemia in our hospital, as well as to record the isolated species of the fungus.
Methods: Prospective epidemiological study, in a tertiary care hospital. The demographic characteristics of all the patients who developed candidaemia were recorded, as well as the underlying diseases, possible predisposing factors, such as presence of central venous catheters, diabetes mellitus, renal failure, etc. The species of *Candida* isolated from blood cultures was identified with Chromagar and API 32C and sensitivity testing was done with the microdilution method.
Results: The study started in January 2006 and is in progress. We report the results of the first 18 months. During this period 49 episodes of candidaemia were recorded in 49 patients. Their mean age was 60 years (range 20 to 90) and 65% of them were male. Twenty-eight were from the surgical, 2 from the urology and 9 from the medical departments and 10 from the ICU. One patient (2%) was neutropenic, 35 (71%) had central venous lines and were receiving parenteral nutrition.

The isolated species were *C. albicans* (17), *C. parapsilosis* (19), *C. glabrata* (8), *C. sake* (1), *C. lusitaniae* (1), *Pichia ohmeri* (1) and *C. tropicalis* (2). The *C. glabrata* strains were all, except one, resistant to fluconazole, two were intermediately and one fully resistant to voriconazole, while the *C. parapsilosis*, *C. lusitaniae*, as well as the *C. albicans* strains were susceptible to all antifungals tested, with the exception of two isolates of *C. parapsilosis* resistant to caspofungin and one strain of *C. albicans* resistant to fluconazole, itraconazole and voriconazole. In addition, there were three strains of *C. parapsilosis* with dose-dependent resistance to fluconazole and two to itraconazole. The isolate of *Pichia ohmeri* was resistant to caspofungin and had dose-dependent resistance to fluconazole and itraconazole. The crude mortality of the patients who developed candidaemia was 42%.
Conclusions: Non-*albicans Candida* species are more common than *C. albicans* in our hospital and *C. parapsilosis* is the prevailing species. The greatest number of cases is recorded in surgical patients and not in patients with neutropenia or other medical underlying diseases. Total parenteral nutrition is very common in patients who develop candidaemia.

P1793 Candidaemia in oncology patients over the past five years

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Objectives: All cases of candidaemia diagnosed in our department over the past five years (2003–2007) were retrospectively analysed to investigate the isolation and distribution rate of *Candida* spp. in blood cultures and to evaluate their antifungal susceptibility.
Methods: Positive blood cultures with BacT/Alert system were examined microscopically directly for yeasts or pseudoyphae and subcultured on Sabouraud agar. *Candida* isolates were screened by germ tube test and identified using API 20 CAUX. Antifungal MIC was performed by using ATB fungus 3 (Biomerieux) and Etest (Biodisk) on RPMI 1640 for the following antifungal: amphotericin B (AB), fluorocytosinec (5F), itraconazole (I), fluconazole (F) and voriconazole (V).
Results: During the study period there were 41 (2.9%) yeast isolates from a total of 1389 patients exhibiting positive blood culture. The underlying disease was a solid tumour in 30 patients and an haematological malignancy in 11 patients. 16 patients were in the ICU at diagnosis with mechanical ventilation. Previous *Candida* colonisation was occurred in 22 patients and central venous catheter in 15 patients. The causative species were: *C. albicans* 26 strains (63%). *C. tropicalis* 6 (14.6%), *C. parapsilosis* 5 (12%), C.glabatra 2 (4.9%)and *C. krusei* 2 (4.9%). *C. parapsilosis* isolated from patients with a central venous catheter. The 2 patients with *C. krusei* had haematological malignancy. Sensitivity rates (%) of *C. albicans* to all antifungal agents were 100 except to F (80). The MIC of the two strains of *C. krusei* to AB was 2 mg/lit.
Conclusions: Candidaemia in oncology patients is predominantly caused by *C. albicans*. The frequency of candidaemia due to *Candida* non-*albicans* is quite high (37%). The highest degree of resistance was observed to fluconazole while voriconazole was very active against all species of *Candida*.

P1794 Epidemiology of nosocomial Candidaemia in a Finnish tertiary care hospital, 1987–2004

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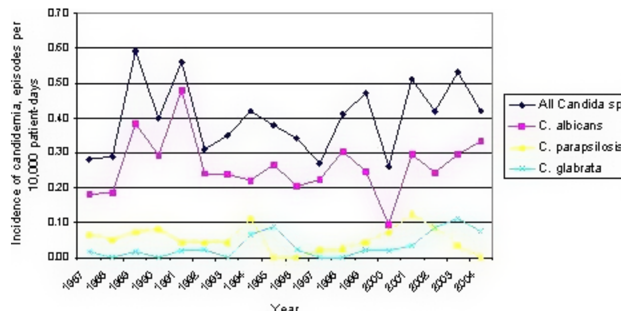
Objectives: Nosocomial bloodstream infections (BSI) caused by *Candida* sp. (i.e. candidaemia) have been shown to be a growing problem in the US. In addition to immunocompromised patients, intensive care and surgical patients are also at risk, mainly because of wide spectrum antibacterial treatment, invasive monitoring and gastrointestinal surgery. We studied the changes in epidemiology of candidaemia by assessing the incidence and outcome of candidaemia as well as causative *Candida* sp. in the Helsinki University Central Hospital (HUCH) during 1987–2004.
Methods: HUCH with 1600 beds serves a population of 1.66 million in Southern Finland. All patients with at least one blood culture positive for *Candida* sp. during 1987–1998 were retrospectively identified from the laboratory logbooks, and the patient charts were reviewed. During 1999–2004, candidaemia episodes were identified through prospective laboratory-based surveillance of nosocomial BSIs, which was carried out as a part of the Finnish Hospital Infection Program. Centers for Disease Control and Prevention definitions for nosocomial BSIs were used.



Overall incidence of candidaemia per 10,000 patient-days as well as incidences by *Candida* sp., Helsinki University Central Hospital, 1987–2004.

Results: A total of 373 episodes of nosocomial candidaemias were observed (average annual incidence, 0.40 per 10,000 patient-days; range by year, 0.27–0.59); no increase in annual incidence was detected (Figure 1). Median age of candidaemia patients was 52 years (range, 0–89 years) and 59% were male. Most common causative species was *C. albicans* (65%), followed by *C. parapsilosis* (13%), *C. glabrata* (9%), *C. krusei* (5%), *C. tropicalis* (3%) and others (13%); no increase in proportion of non-*albicans* sp. was observed. During 1987–1998, the proportion of intensive care patients with candidaemia varied between 24–27%, but by the period of years 1999–2004 it increased to 44%. The increase was observed in neonatal (15–21%) and surgical (46–55%) patients but not in patients with haematologic malignancy, whose proportion decreased (21–11%). Most (68%) patients with candidaemia had central-lines. The one-month case fatality was 31%; no changes over the six-year periods were observed (range, 30–32%).
Conclusions: During the 18-year study period, no increasing trend in the incidence of nosocomial candidaemia was detected and no shift towards non-*albicans* sp. occurred. Crude mortality remained high. The changes in the patients groups at risk may reflect differences in prevention strategies which need to be analysed for further improvement of prevention.

P1795 Epidemiology, risk factors, and clinical outcome of candidaemia in a tertiary care hospital in Korea

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Objectives: *Candida* spp. is the fourth most common cause of bloodstream infections, and *C. albicans* is the most common species. However, changes in *Candida* spp. epidemiology have been reported. The purpose of this study is to determine the epidemiology, risk factors and outcome of *C. albicans* (CAB) and non-*albicans* BSI (CNAB) in a tertiary care centre in Korea.
Methods: All inpatient, adult candidaemia cases between 2003 and 2006 were identified at our centre. Demographic characteristics, risk factors, time to antifungal agent, and mortality within 30 days of culture were obtained from retrospective record review.
Results: A total of 300 episodes of candidaemia occurred in 101 patients over the 4-year period. The distributions of species were as follows: 48 *C. albicans*, 23 *C. tropicalis*, 13 *C. glabrata*, 12 *C. parapsilosis*, 4 unclassified spp., and 1 *C. krusei*. There was significant difference between the incidences of CAB (32.4%) and CNAB (67.6%) in 2006 ($p = 0.021$). There was no significant difference in demographic

characteristics, risk factors, underlying diseases, time to antifungal therapy, and clinical outcome between CAB and CNAB. Of 73 (72.3%) central venous catheters at the culture date, 43 (53.9%) were removed. Catheter related candidaemia which proven by tip culture was 13 cases. The 57 (56.4%) of these 101 cases were cultured at ICU. Of 101 patients, the median age was 66 year (range, 32–86 year); 65.3% were males. Median length of stay before the first positive culture date was 22 days, and APACHE II score at culture date was 18. Antifungal therapy had been done for 59 patients (58.4%) after median 4 days of culture. Fluconazole was primarily used in 45 (76.3%) patients, and switched to amphotericin B (9) as second-line drug. Reasons for absence of antifungal therapy were: death or hopelessly discharged before culture result (24), clinical improvement or felt to contamination (13), and unknown (5). Overall, 52 patients (51.5%) died. Attributable mortality of candidaemia was significantly higher in untreated cases than treated (59.5% vs. 22.0%, p = 0.002).
Conclusion: Currently CNAB is increasing without specific risk factors in our centre. Strategies should be needed to decrease the incidence of untreated candidaemia, which related with high mortality. Educational efforts should be also needed to reinforce the clinical significance of candidaemia, early empiric therapy for high-risk patients, and earlier detection of candidaemia.

P1796 Mortality, hospital stay and costs associated with candidaemia in major oesophagus, stomach, duodenum and small and large intestinal procedures

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Objectives: To describe the mortality and cost consequences of candidaemia in major oesophagus, stomach, duodenum and small and large intestinal procedures.
Methods: The Premier's Perspective Comparative Database was used to assess mortality, length of stay (LOS) and costs for all oesophagus, stomach, duodenum, small and large intestine DRGs between January 2005 and December 2006 in 296 U.S. hospitals. Patients with and without candidaemia were compared on mortality, APR severity of illness and risk of mortality subclasses, LOS and costs. Candidaemia was defined as an ICD-9 discharge diagnosis of 112.5 (disseminated candidaemia). A General Linear Model (GLM) assessed the effect of candidaemia on total costs after controlling for confounders. Costs are reported in U.S. dollars.

	Candidaemia (n = 283)	w/o Candidaemia (n = 5653)
Mortality	24.0	17.5
APR Risk of mortality subclass		
% Mild	0.0	9.0
% Moderate	2.8	14.9
% Severe	18.7	28.3
% Extreme	78.5	47.9
Mean LOS (SD)	37.8 (34.0)	23.5 (19.9)
Mean total cost $ (SD)	$78,715 (78,823)	$50,667 (45,280)
Mean antifungal cost $ (SD)	$3,130 (5,018)	$1,221 (2,360)

Results: Caucasian ethnicity and age in the candidaemia and non-candidaemia groups were significantly different (% white = 60.1 & 68.9, respectively, p = 0.0019; mean age = 67.7 & 65.1 years, p= 0.0037); there were no significant differences in sex (% female = 59.0 & 55.9%). Candidaemia was associated with a 63.8% increase in the percent of patients with extreme severity APR risk of mortality, 37.1% greater in-hospital mortality, 61.2% longer mean LOS, and 55.4% higher total costs. Anti-fungal drug treatment represents 4.0% and 2.4% of total costs for patients with and w/o candidaemia, respectively. In unadjusted analyses, differences in mortality were not statistically significant; all other table differences were significant (p < 0.0001). The presence of candidaemia was associated with a significant increase in total costs ($15,727; F = 34.4; p < 0.0001) after controlling for age, ethnicity, APR severity risk, APR mortality risk and discharge status.
Conclusion: Candidaemia extracts a high toll in LOS and costs; anti-fungal costs represent a minor portion of the total. This highlights the importance of improved prevention and treatment of candidaemia in patients undergoing major oesophagus, stomach, duodenum and small and large intestinal procedures.

P1797 Relationship between candidaemia and prior colonisation with *Candida* species in critically ill adults

J. Troughton, G. Browne, D. McAuley, J. Walker, R. McMullan (Belfast, UK)

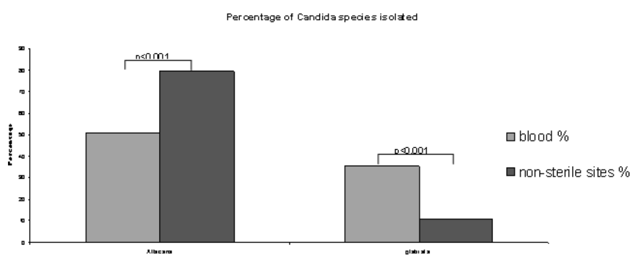
Objectives: Pre-emptive antifungal therapy in the critical care setting is based upon the species and drug susceptibilities of *Candida* species isolated from at least 2 non-contiguous skin and mucosal sites of high risk patients. However the relationship between colonising *Candida* spp. and those causing invasive disease is not definitively established. The aim of this study was to determine the proportion of patients with candidaemia who had prior *Candida* colonisation of 2 non-contiguous anatomic sites and to describe the species involved.
Methods: Prospectively collected laboratory records of blood cultures from ICU patients at a University hospital in Belfast were analysed retrospectively. Patients with *Candida* spp. cultured from blood in alternate years from 1999–2007 were selected and data collected on cultures from non-sterile sites in this cohort. A per-isolate analysis was conducted.
Results: A total of 49 patients had confirmed candidaemia; since four of these were mixed candidaemias there were 53 blood isolates, in total. Overall, for 19 (36%) of the blood isolates, the same species of *Candida* was recovered from at least 2 non-sterile non-contiguous sites; for 18 (34%) of the blood isolates, the same species was recovered from only one non-sterile site. Sixteen (30%) blood isolates were recovered from 15 patients who had no *Candida* spp. recovered from non-sterile sites. *C. glabrata* candidaemia was significantly less likely to be preceded by positive non-sterile site cultures than non-*glabrata* species (15/18 vs 9/35, p < 0.0001). Moreover, of three patients in whom there was discordance between blood and non-sterile site isolates all had *C. glabrata* candidaemia.
Conclusions: These data suggest that the current pre-emptive prescribing practice that is widely adopted in ICUs may be inadequate, since almost two thirds of candidemic patients in this series would not have received an antifungal drug on this basis alone. Moreover, *C. glabrata*, which often exhibits reduced susceptibility to fluconazole, is especially likely to present with candidaemia without prior colonisation.

P1798 The relationship between *Candida* species isolated from non-sterile sites and blood in critically ill adults

J. Troughton, G. Browne, D. McAuley, J. Walker, R. McMullan (Belfast, UK)

Objectives: Pre-emptive prescribing of antifungal drugs has become a widespread therapeutic strategy in critically ill adults; antifungal drug selection is based upon the *Candida* species isolated from patients' non-sterile sites and their drug susceptibility. However, few data are available that convincingly prove a consistent relationship between isolates recovered from skin and mucosal anatomic sites and those responsible for invasive disease. The aim of this study was to investigate: firstly, whether the species distribution of blood isolates was significantly different to those recovered from non-sterile sites, and, secondly whether there was a difference in the proportion of fluconazole (fcz) sensitive isolates between blood and non-sterile sites.
Methods: Prospectively collected laboratory records of blood cultures and non-sterile site specimens from ICU patients at a University hospital

in Belfast were analysed retrospectively. Data from alternate years from 1999–2007 were evaluated and included if a *Candida* sp. had been cultured. Only the first isolate of any species from a given site (non-sterile site or blood) from each patient was included.



Results: In total, 51 blood cultures and 1002 non-sterile specimens from 927 patients were evaluated. From blood, 51% isolates were *C. albicans*, 35.3% *C. glabrata* and 13.7% non-*albicans*/non-*glabrata* species. From non-sterile sites, 79.3% isolates were *C. albicans*, 10.4% *C. glabrata* and 10.4% non-*albicans*/non-*glabrata* species. Overall, a significantly higher proportion of *Candida* spp. from non-sterile sites were *C. albicans* compared with blood; and a significantly higher proportion of *Candida* spp. from blood were *C. glabrata* compared with non-sterile sites (see graph). There was no difference in the proportion of non-*albicans*/non-*glabrata* species between blood and non-sterile sites. A significantly greater proportion of isolates from non-sterile sites were fcz-sensitive compared with blood ($p < 0.001$).

Conclusions: *Candida* spp. isolated from non-sterile sites were not representative of isolates from blood, either in terms of species distribution or fcz sensitivity. This brings into question the validity of the premise on which extant pre-emptive prescribing is based.

P1799 Invasive candidiasis, *Candida* colonisation and antifungal treatment in intensive care patients after cardiothoracic surgery

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Objectives: To improve the diagnosis, the timing of diagnostic procedures and antifungal treatment of invasive *Candida* infections in intensive care patients, a prospective surveillance study was performed at our cardiothoracic intensive care unit (ICU) of the Medical University of Vienna.

Methods: Patients admitted to the cardiothoracic ICU between December 2006 and November 2007 were enrolled into the study. Two times weekly surveillance cultures ($n = 2413$) of inguinal swabs (17%), axillary swabs (17%), pharyngeal swabs (17%), urine (14%), nasal swabs (13%), bronchial lavage (11%), surgical wounds (4%) and anal swabs (2%) were taken. At each time point material from at least five different body sites was analysed for presence of *Candida*. The Candida Colonization Index (CI) was calculated for each patient by the number of *Candida* positive samples per all samples. Patients were grouped as follows: no colonisation ($CI \leqslant 0.2$; $n = 19$), colonisation ($CI > 0.2 < 0.6$; $n = 30$) and severe colonisation ($CI \geqslant 0.6$; $n = 36$). As risk factors time of stay on the ICU, number and type of surgeries, sex and age of patients and start of antifungal treatment were investigated.

Results: A total of 85 patients were enrolled into the study, the overall mortality was 39.2%. The different types of surgeries included valve replacement, aortocoronary bypass, heart or lung transplantation, implantation of left ventricular assist device or artificial heart with a mean of 2.1 surgeries per patient. During the first four weeks on ICU *Candida albicans* was the predominating pathogen (39 to 49% of all cultures). The percentage of Non-*albicans* species, particularly *Candida parapsilosis* and *Candida glabrata* increased over time. Sex, age of patients, the type of surgery and the start of antifungal treatment were no significant risk factors for severe colonisation. A significant difference between colonised and severly colonised patients was detected for the time of stay on ICU (median= 23 days versus 48 days; $p < 0.001$) and the number of surgeries (median= 1.7 versus 2.7; $p = 0.02$). All patients who developed Candidaemia ($n = 4$) were previously severly colonised with *Candida*. The mortality rate in multifocal colonised patients was 50%.

Conclusion: Prolonged stay on ICU and number of surgical interventions >2 predicted for severe colonisation in patients at a cardiothoracic ICU. Candidaemia was exclusively detected in patients who were previously severly colonised with *Candida*.

P1800 Incidence of newer antifungal consumption and incidence of Candidaemia in an adult intensive care unit

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Objectives: A large proportion of nosocomial infections are acquired in intensive care units (ICU). *Candida* spp. are the leading non-bacterial nosocomial pathogens, and candidaemia carries a high risk of mortality in critically ill patients. *Candida* spp. are responsible for the majority of nosocomial fungal infections.

The study objectives were to describe the local epidemiology of candidaemia in the ICU of a tertiary care hospital, to analyse the sensitivity of *Candida* strains to the antifungal compounds and to describe the consumption of the newer antifungals compounds.

Methods: Hospital medical records of adult patients with candidaemia were reviewed from January 2000 to December 2006. We analysed the candidaemia episodes at the adult ICU of the institution. Demographic information and overall mortality were retrieved. We tested the susceptibility of *Candida* strains isolated during the last five years in our institution.

Results: A total of 212 episodes of candidaemia occurred in the institution. 49 (23%) developed in the ICU: 67% in surgical ICU and 33% in medical ICU. The median age of patients with candidaemia was 54.7 years; 27 (55%) were males. The mortality rate was 39%. *Candida albicans* was responsible for 71% ($n = 35$) of infections acquired in ICUs. Non-*albicans Candida* species (29%) were equally distributed throughout the study period. *C. glabrata* was identified in 8 episodes (16%), followed by *C. parapsilosis* in 2 (4%), *C. tropicalis* in 3 (6%), and unspecified *Candida* species in 1 (2%). During the study period no switch was noted in the distribution of *C. albicans* and non-*albicans Candida* species. The range of candidaemia incidence varied from 0.6 to 3.4 episodes/1000 patients throughout the study period. Most strains of *C. albicans* (94%) remained highly sensitive to fluconazole and all the newer antifungals tested. A decrease of the use of Amphotericin B was observed. A trend to increase the consumption of the newer antifungal compounds was observed from 2,200 DDD/year to 3,000 DDD/year.

Conclusion: Fungal infection remains a severe disease associated with high crude mortality. We observed a variable incidence of candidaemia during the study period. *C. albicans* was the most commonly isolated species. A trend to increase the use of the newer antifungal compounds consumption was observed but not a decrease in candidaemia incidence.

P1801 Species distribution and resistance profile to antifungal agents of *Candida* bloodstream infections in ICU-patients. Emergence of voriconazole resistance

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Objectives: To study the *Candida* species caused fungaemia in ICU-patients and their in vitro resistance profile to commonly used antifungal agents.

Methods: during a two year time period (2005–2007) we examined 84 fungal isolates recovered from positive blood cultures of equal numbered patients hospitalised in the three ICUs of our hospital.

The yeasts were differentiated by using germ-tube test and by the automated system VITEK 2 (Biomerieux, France). Sensitivity testing was performed by the system Fungus 3 (Biomerieux, France) and confirmed by the determination of MIC values of the examined antifungal

agents by E-test strips (AB Biodisk, Solna, Sweden) in accordance to the manufacturer's recommendations. MICs were noted after 24 h of incubation. MICs (mg/L) at which 90% of the strains were inhibited were 1 for amphotericin B, 32 for 5-flucytosine, 8 for fluconazole and 0.25 for voriconazole.

Results: The more often encountered species was *Candida parapsilosis* n = 55 (65.5%), followed by *C. albicans* n = 17 (20.2%), *C. glabrata* n = 5 (5.9%), *C. famata* n = 4 (4.6%), *C. lusitaniae* n = 2 (2.3%) and *C. utilis* n = 1 (1.2%).

No resistance to amphotericin B was observed to any kind of *Candida* spp. The resistance level of the examined fungal isolates to fluconazole was 14.3%, to itraconazole 19% and to 5-flucytosine 51.3%. The second year two (2.3%) *C. albicans* isolates were found resistant to voriconazole, for the first time in our hospital and one isolate of *C. albicans* with decreased sensitivity (dose-depended) to the same antifungal agent.

Conclusion: The most prevalent isolated species recovered from blood stream infections of ICU-patients was *Candida parapsilosis*. Resistance to voriconazole emerged for the first time in our hospital making the treatment of fungal infections due to *C. albicans* species, more difficult. Amphotericin B remains an agent with very good activity for the treatment of *Candida* blood stream infections.

P1802 Distribution of *Candida* species in a neonatal intensive care unit before and after the introduction of fluconazole prophylaxis for very low birth-weight babies

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Objectives: On 1st October 2003, a protocol for administering prophylactic fluconazole therapy to very low birth weight babies with identified risk factors was implemented at our institution. Accordingly, the potential for this to result in a species-shift towards in fluconazole resistant species such as *C. glabrata* and *C. krusei* merits epidemiological surveillance. The aim of this study was to investigate whether any species shift had occurred in association with the protocol.

Methods: Prospectively collected data from the mycology laboratory records were analysed retrospectively. All *Candida* isolates from the Neonatal Intensive Care Unit (NICU) were included in the analysis. Data from two time periods were compared; the first was from 1st October 1999 to 30th September 2003 and the second from 1st October 2003 to 30th September 2007. Isolates from each period were classified as "*Candida albicans*" or "non-*albicans Candida*" from either "blood" or "other" sites. Differences observed between the two time periods were tested for significance using Fisher's exact test.

Results: Prior to the implementation of the protocol there were 93 isolates of *Candida*. Of these 68 were *Candida albicans* (16 blood, 52 other sites) and 25 were non-*albicans Candida* (5 blood and 20 other sites). After the application of the protocol there were 65 isolates of *Candida*; 62 isolates of *Candida albicans* (13 blood, 49 other sites) and 3 non-*albicans Candida* (0 blood and 3 other sites). There was a significant trend towards increasing *Candida albicans* versus non-*albicans* isolates from all sites (p = 0.0002). This may be explained by a trend in isolates from non-sterile sites (p = 0.002); there was no significant change in species distribution of blood isolates (p = 0.1317).

Conclusions: We did not observe an increase in non-*albicans Candida* following the implementation of the fluconazole prophylaxis protocol. In fact, surprisingly, there was a significant reduction in the proportion of non-*albicans Candida*. Furthermore, there were no isolates of fluconazole resistant *Candida* spp. recovered in either four year period.

P1803 Impact of highly active antiretroviral therapy and *Candida* spp. oral colonisation in AIDS patients in a Brazilian tertiary-care university hospital

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Objectives: To evaluate *Candida* spp. oral colonisation in AIDS patients under highly active antiretroviral therapy (HAART).

Methods: prospective cohort study of 331 patients assisted in a University tertiary care hospital. An oral swab was performed and inoculated in CHROMagar™ *Candida*. *Candida* species were determined using ID32C and serotypes of *C. albicans* were identified by *Candida* check test. Antifungal susceptibility testing, by microdilution, was performed in all isolates for 5-FC, azoles, amphotericin B, nistatin. Clinical and laboratorial data were collected to identify the association with *Candida* colonisation. Karyotyping was done in *C. albicans* isolates.

Results: 161 *Candida* spp. isolates were detected in 147 (44.4%) patients and *C. albicans* represented 137(85%) of the isolates followed by *C. glabrata* (29%); *C. tropicalis* (16.7%); *C. norvegensis* (12.5%); *C. dubliniensis* and *C. krusei* (8.3%). Seven patients had oral candidiasis at the moment of sampling the oral cavity. All *C. albicans* were susceptible to all the tested antifungal drugs. Among the non-*albicans*, six isolates were SDD to fluconazole; nine to itraconazole and seven to cetoconazol. Azole resistance was not detected. Karyotyping showed 15 different profiles in *C. albicans* serotype A (117strais) and 90% of *C. albicans* serotype B (18/20 strains) had the same DNA profile. The isolation of *Candida* was significantly higher in patients with detectable viral load (83/147) (p = 0.0002) and CD4+ T-lymphocyte <200 cells/mm^3 (30/83)(p = 0.0003); the median CD4+T cells/mm^3 in the colonised group (374.4) was significantly lower than the non-colonized (471.5) (p = 0.003). The incidence of *Candida* in oral cavity was not different in patients under HAART with or without protease inhibitor (PI) (colonized: 78/133; non colonised: 91/170) (p = 0.60)

Conclusion: Oral *Candida* colonisation in patients under HAART was associated with low CD4+ T cells/mm^3 and failure response to HAART. Despite PI had in vitro antifungal activity, the use of this class of antiretroviral did not influence the presence of *Candida* in the oral cavity of AIDS patients.

P1804 Lower saliva flow rates are associated with oral affection of *Candida* sp. after radiation therapy

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Objectives: Xerostomia is one of the late side effects of radiation therapy in patients with head and neck cancer associated with great impairment of quality of life. Radiation therapy can also predispose to oral candidal colonisation of the oral mucosa in the immuncompromised patients. This study was focused on correlation between the total unstimulated saliva flow rate and the fungal colonisation of the oral cavity.

Methods: In 30 patients who had radiation therapy the unstimulated saliva flow rate was analysed during five minutes. According to the measured rates the results were divided into pronounced xerostomia (unstimulated whole saliva flow rate = 0.0 ml/min), xerostomia (unstimulated whole saliva flow rate $\leqslant$0.1 ml/min) and hyposalivation (unstimulated whole saliva flow rate >0.1–<0.25 ml/min). After determination of the saliva flow rate 2 ml sodium chloride were used to rinse out the mouth for 20 seconds. *Candida* colonisation was defined using sabouraud agar and identified using API *Candida* (biomerieux) in the mouth rinsing water of patients.

Results: Pronounced Xerostomia was detected in 17 patients (median = 10^6 cfu; range 0–10^7 cfu), xerostomia in 11 patients (median = 10^5 cfu; range 0–10^6 cfu) and hyposalivation in 5 patients (median = 0 cfu; range 0–10^6). Spearman's correlation coefficient was –0.331 (p = 0.06). In our collective there was no patient having a normal saliva flow rate. 11 *Candida*-species were identified as *Candida albicans*, 5 as *Candida famata*, 4 as *Candida glabrata*, 1 as *Candida tropicalis*, 1 as *Candida dubliniensis* and 3 as *Saccharomyces cerevisiae*. In 8 patients no *Candida*-species were detected.

Conclusion: Lower saliva flow rates correlate with a higher risk for *Candida* affection in patients after radiation therapy.

P1805 The economic and service impact of invasive candidiasis in the UK

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Background: Invasive candidiasis (IC) is a serious nosocomial infection likely to impact length of stay (LOS) and medical resource use in hospital. We examined the consequences of acquiring IC on healthcare resources to estimate their potential burden on the UK healthcare system.
Methods: We utilised data from the UK Clinical Hospital Knowledge System (CHKS), comprising national hospital inpatient activity and the Secondary Linked Inpatient to Mortality Dataset (SLIM) comprising the resident population of Cardiff and the Vale of Glamorgan (population of 424,000 – 2001 estimate). Patients with a diagnosis of invasive candidiasis as defined by selected ICD-10 codes (B37.1, B37.5, B37.6, B37.7, B37.8, B37.9) during 1995–2005 in SLIM and during 2005–2006 in CHKS were identified from inpatient admissions. A matched-pair analysis was performed by selecting control patients without IC matched on age, gender, primary diagnosis and procedure. LOS, mean admission costs and mortality were compared to determine the excess burden of IC.
Results: The majority of admissions had a primary diagnosis other than IC. From SLIM 275 IC patients were identified and 241 (96%) of these with a secondary diagnosis of IC were matched. Patients with a diagnosis of IC had increased medical resource use: longer mean LOS (13.3 days vs. 8 days), increased mean admission cost (£1,879 vs. £1,616) and increased hospital mortality (11% vs. 5%). From CHKS 6,105 (95.1%) patients with secondary diagnosis of IC were matched to patients without IC. Again, patients with a diagnosis of IC had increased mean LOS (16.6 vs. 5.6 days), higher mean admission costs (£1,739 vs. £1,531) and higher in-hospital mortality (11.3% vs. 5.0%). Patients with a diagnosis of IC who were in the ICU had longer mean overall hospital LOS (40.3 v 20.8 days) and increased mean LOS in ICU compared to non-IC patients (16.7 days v. 7.5 days).
Conclusions: Patients with IC have overall longer LOS in hospital and in the ICU compared to patients without IC, resulting in both service and economic impacts. These data probably underestimate the true IC impact as it does not account for individuals with IC coded as general septicaemia. The difference in mean costs is also likely to be higher when excess bed day HRG costs and ICU per diem costs are included.

CHKS matched pair analysis results

	Cases (n = 6,105)	Controls (n = 6,105)
Mean (sd) LOS		
Total hospital days	16.6 (33.2)	5.6 (12.5)
– for patients with ICU/HDU stay	40.3 (40.3)	20.8 (28.3)
ICU days	16.7 (18.8)	7.5 (15.9)
Total hospital mortality* (%)	11.3%	5.0%
Mean (sd) admission cost	£1,739 (1,711)	£1,531 (1,235)

*p < 0.05 cases vs. controls.

P1806 Review of vulvovaginal candidiasis isolated in a Spanish hospital in a two-year period (2005–2007)

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Objectives: Better understanding of the aetiologic agent of all vulvovaginal candidiasis (VVC) isolated in our hospital during a two-year period.
Methods: 2052 samples received in H.U. Miguel Servet from June 2005 to June 2007 belonging to 1912 women between ages 1 and 94 with a diagnose of VVC were reviewed.
Samples were cultured in OCCA (Oxoid® Chromogenic *Candida* Agar) and Oxoid® Sheep Blood Agar.
Initial chromogenic identification of the different yeast species was confirmed by biochemical tests using API 20 C AUX® Biomerieux.
Results: With a prevalence of 86.5% *C. albicans* remains the dominant species non-*albicans* species more frequently found was *C. glabrata* (10.3%), followed by *C. krusei* (1.3%), *C. parapsilosis* (0.8%), *C. tropicalis* (0.6%), *C. kefyr* (0.1%), *C. lusitaniae* (0.1%) and *Saccharomyces cerevisiae* (0.2%).
In our reviewed samples, 140 patients had some recurrence, of which 5% had at least two or three episodes per year, and 0.3% had a chronic VVC (four or more episodes per year). Also, a higher incidence of VVC was observed between the second and the fourth decade of life, followed by a sharp decline in the fifth decade.
Conclusions: In the last years there has been a rise in the share of VVC attributable to non-*albicans Candida* species, specially *C. glabrata* and *C. krusei*, which are less susceptible to azole-derived antifungal agents, and therefore more difficult to eradicate.
In studies from other geographical regions, non-*albicans* yeasts isolated in VVC patients show a different distribution pattern. This fact stresses the importance of knowing the aetiological agents in each hospital and in each population in order to obtain the most precise diagnosis and treatment.

P1807 Genital candidiasis in prepubertal and pubertal girls

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Objectives: Vulvovaginal candidiasis is a frequent infection in women, especially of reproductive age. Although insufficiently studied in children, its prevalence is lower in children of prepubertal or pubertal age. High estrogen levels were associated with the presence of yeasts in vaginal samples of symptomatic women while low estrogen levels during childhood result in a lower incidence in prepubertal girls. This study was conducted in order to determine the prevalence of culture-positive candida infection in children with genital symptoms in our hospital.
Methods: We obtained and cultured 439 vaginal samples from an equal number of Virgo girls aged from 1 to 18 years old presenting to our hospital from May 2005 to October 2007 with signs and symptoms of vulvovaginitis. In order to isolate *Candida* spp., all samples were cultured on Sabouraud Dextrose Agar (Conda, Madrid, Spain) for 24–48 hours at 37ºC. *Candida* spp. were identified on the basis of the macroscopic appearance of colonies, direct microscopy of wet mount and Gram-stained specimens. Finally, the identification to the species level was completed using the API System ID 32 C (BioMerieux, France).
Results: Out of the 439 vaginal samples studied, 40 samples yielded *Candida* spp., which represent the 9.1% of the samples. In particular, we isolated 27 *C. albicans* (67.5%), 7 *C. glabrata* (17.5%), 5 *C. tropicalis* (12.5%) and 1 *C. sake* (2.5%). When we attempted to sort the 40 cases with *Candida* by age groups, in the 1–5 years age group we included 2 samples (5.0%), in the 6–10 years group 1 sample (2.5%), in the 11–15 years group 7 samples (17.5%) and in the 16–18 years group 30 samples (75.0%).
Conclusion: In girls less than 11 years old we observed a low prevalence of *Candida* spp. In contrast, in pubertal girls a higher incidence of *Candida* spp. was noted, probably because of the rise in estrogen levels. *Candida albicans* was the most frequently isolated yeast from vulvovaginal candidiasis in our study group.

Atypical mycobacteria

P1808 Pulmonary *Mycobacterium simiae* infection. Comparison with pulmonary tuberculosis

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Objectives: *M. simiae* is endemic to Israel. Recently, we observed an emergence of *M. simiae* as the most frequently isolated non-tuberculous

mycobacterium in respiratory specimens in our institution, followed by *M. fortuitum*, *Mycobacterium-avium*-complex, and *M. chelonae*. The distinction of *M. simiae* respiratory infection from pulmonary tuberculosis on the basis of simple clinical and radiological features has significant therapeutic and infection control importance. The objective of this study was to identify clinical and radiological features distinguishing *M. simiae* respiratory infection from pulmonary tuberculosis.

Methods: The database of the Laboratory of Clinical Microbiology of a major tertiary medical centre was searched for all patients with culture-positive *M. simiae* respiratory infection from 1996 to 2004. Data on demographics, underlying conditions, and clinical and radiological findings were collected and compared with consecutive patients who attended two tuberculosis centres in the same geographic area from 1999 to 2005.

Results: The *M. simiae* group included 102 patients and the tuberculosis group, 121. The patients in the *M. simiae* group were significantly older (mean 69 ±16 years vs 47±21 years, $p=0.000$), with a female predominance (62% vs 45%, $p=0.008$). Only 4% were of Ethiopian origin compared to 25% of the tuberculosis group ($p=0.000$). *M. simiae* infection was associated with significantly higher rates of smoking history, underlying chronic obstructive pulmonary disease, diabetes mellitus, ischemic heart disease, and malignancy, and treatment with immunosuppressive drugs. HIV infection was found in 10% of the patients with tuberculosis and in none of the patients with *M. simiae* infection ($p=0.001$). In the *M. simiae* group, clinical signs were blunted, and noncavitary infiltrates in the lower/middle lobes were observed more often on chest x-ray.

Conclusions: HIV-negative patients with *M. simiae* respiratory infection are distinguishable from patients with pulmonary tuberculosis by several demographic, clinical, and radiological features. These findings have important diagnostic and therapeutic implications.

P1809 Are mycobacteria present in colon biopsies from patients with early inflammatory bowel disease?

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Objectives: Crohn's disease is a chronic condition with inflammation in the intestines. A strong correlation between the presence of *Mycobacterium avium* ssp. *paratuberculosis* and Crohn's disease has previously been inferred, and a far higher incidence of *M. a. paratuberculosis* PCR positivity has been shown in the gut of Crohn's patients than in healthy people. It is, however, not clear if *M. a. paratuberculosis* has an aetiological impact in the pathogenesis of Crohn's disease, or if it is just a coincidence in that Crohn's patients have an increased predisposition for colonisation by *M. a. paratuberculosis*. We extended this question to ask if there is an increased presence of mycobacteria in general in colon biopsies from patients with inflammatory bowel disease (IBD; Crohn's disease and ulcerous colitis) as compared to healthy people. To facilitate the mycobacterial detection we used cultivation in enriched media in combination with direct detection by PCR.

Methods: Biopsies were prospectively taken from inflammatory lesions from all sections of the colon from 400 patients with suspected IBD and 140 controls submitted to colonoscopy (the IBSEN II study). One hundred and fifty control patients were also included. Patients were later grouped according to IBD status: Crohn's disease, ulcerous colitis, other condition or healthy colon. The biopsies were investigated for the presence of mycobacteria by 16S rRNA gene PCR analysis and cultivation in the BACTEC MGIT 960 continuous culture system. The specimens were cultivated in mycobactin-enriched BBLTM Middlebrook 7H9 medium. Mycobacterial strains isolated were identified by species-specific hybridisation, *M. a. paratuberculosis*-specific IS900 PCR and 16S rDNA PCR sequencing and blast analysis. Specimens from selected patients were subjected to metagenomics analysis to describe the global gut flora.

Results: Among the samples from the 540 patients investigated, only 1 patient was mycobacterial culture positive and IS900 PCR positive. This patient had a liver transplant after primary sclerosing cholangitis and contracted Crohn's disease. Mycobacteria were so far not detected in biopsies from the healthy controls.

Conclusion: Taken together the findings from this study will generate novel knowledge on the occurrence of mycobacteria in the human gut. This will extend the basis for defining a putative aetiological role of mycobacteria in early IBD, with special emphasis on Crohn's disease.

P1810 Utilising clinical parameters to differentiate mycobacteraemia from *Mycobacterium tuberculosis* v.s. from *Mycobacterium avium* complex on the day of blood culture signal detection

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Objectives: Disseminated MAC (DMAC) and TB (DMTB) share many clinical features and treatment of these two entities are different. Not all mycobacterial laboratories, especially in developing countries, are equipped to perform rapid, same-day molecular identification of the grown mycobacteria. We sought to identify clinical predictors to aid clinicians in species prediction on the day of positive signals.

Methods: At our hospital, blood cultures for mycobacteria and fungi are incubated in Bactec MYCO/F bottles. Content from positive-signals bottles is stained for acid-fast bacilli (AFB) and subcultured. Specimens with AFB are subjected to commercial, and, when deemed necessary, manual, molecular identification to the species level. We analysed certain clinical parameters that may correlate with disseminated TB (DTB) or MAC (DMAC).

Results: A total of 2,040 blood specimens were sent for mycobacterial and fungal isolation by Bactec MYCO/F medium during Jan 2005-Nov 2007. TB and MAC were isolated in 109 patients. Clinical data were available in 87 of them. The prevalence of mycobacteraemia from MTB is higher. Sensitivity and specificity of AFB smear from Bactec content for mycobacterial identification are 92% and 96% respectively. Smear of content of Bactec MYCO/F medium, which gives positive signals, can accurately predict growth of mycobacterium. Most patients had low CD4 cells (mean 89, median 48). The CD4 in patients with DMAC was significantly lower ($p<0.05$). Mycobacteraemia from MAC gave positive signals faster than that from MTB. Patients with DTB tended to have abnormal chest x-ray and higher levels of serum alkaline phosphatase. DMAC had normal chest x-ray or had pulmonary infiltrates clearly attributable to another opportunistic agent.

Demographic, baseline CD4, laboratory findings and time until positive signal of Bactec M/F LYTIC

Characteristics	TB	MAC	
Number of patients (%)	55	45	
Sex (M:F)	29:20	27:11	
Age (years)			
Median	36	32	
Range	22–61	25–63	
CD4 (cells/mm^3)			
Median	62	37	$p<0.05$
Mean	103	53	
Range	3–389	1–143	
Alkaline phosphatase (U/L)	370	156	$p<0.05$
Abnormal chest film	30/32	3/26 (PCP=1, old pulmonary TB=2)	
Days for detection of positive signal from Bactec (days)			
Median	27	12	$p<0.05$
Mean	28	13	
Range	10–46	2–42	

From total 87 patients.

Conclusion: In our country, with high incidence of TB, prevalence of mycobacteraemia from DMTB in advanced HIV-infected patients is higher than that from DMAC. All patients with CD4 < 60 who had either normal chest x-ray or known lung lesions from other causes, whose blood culture give positive signals faster than 15 days, have DMAC and not DTB. Our data can be used for selection of an appropriate initial treatment regimen in HIV-infected patients with suspected disseminated mycobacterial infection.

P1811 Prevalence of atypical mycobacteriosis in aquarium fish in northern Italy

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Objectives: MOTT or NTM include members of genus *Mycobacterium* not belonging to *M. tuberculosis* complex. NTM are ubiquitous, opportunist and some of them like *Mycobacterium* marinum, *M. chelonae* and *M. fortuitum* are considered zoonotic agents. They are often founded in aquarium environments and may cause chronic granulomatous diseases in fish and cutaneous infections in fish handlers. Nevertheless the presence and distribution of mycobacterial species in aquarium fish and their environment has not been adequately explored yet.
The present study reports the prevalence of mycobacteria in aquarium fish of Northern Italy sent to the Istituto Zooprofilattico Sperimentale of Piemonte, Liguria e Valle d'Aosta from 2005 to 2007.
Methods: 525 aquarium fish, both from fresh and salt water, were subjected to anatomopathological examination and mycobacterial isolation. When positive growth was obtained the colonies were subjected to biochemical identification. Granulomatous lesions identified macroscopically were evaluated by Haematoxylin and Eosin (HE), acid-fast (Ziehl-Neelsen – ZN) and Auramine-Rhodamine staining and by Immunohistochemistry with a polyclonal antibody against *Mycobacterium bovis*.
Results: 226 out of 525 (43%) resulted positive for *Mycobacterium* spp. We identifies the following species: 100 *M. chelonae* complex (59 *M. chelonae* and 41 *M. abscessus*); 81 *M. fortuitum* complex (42 *M. peregrinum* and 39 *M. fortuitum*); 41 *M. gordonae*; 3 *M. marinum* and 1 *M. scrofulaceum*.
Microscopically granulomas presented a central eosinophilic area (necrosis) surrounded by inflammatory cells and enclosed by a thin capsule. All granulomas resulted ZN positive and the other staining confirmed these results.
Conclusion: Our data indicate that MOTT are densely diffuse in aquarium and that they could represent a possible source of infection for both aquarium fish and immunodeficient fish handlers. Our results are in agreement with the recent mycobacterial bibliography.

P1812 Delayed diagnosis of mycobacterial skin and soft tissue infections in non-immunocompromised hosts

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The awareness of atypical pathogens in skin and soft tissue infections is low and therefore the diagnosis is often delayed. We report three cases of mycobacterial skin and soft tissue infections in normal hosts. The diagnosis was delayed for 3–10 months in all 3 cases.
Our goal is to raise the awareness of atypical pathogens in non-immunocompromised patients with prolonged skin and soft tissue infections, particularly, if there was an injury with biological materials.
Case 1: A 58 year-old florist injured herself with a rose thorn. Despite antibiotic treatment and surgery the wound continued to suppurate and after 3 months she developed a tendosynovitis. As steroid infiltration did not result in improvement, there was another surgical approach. Histology revealed a granulomatous inflammation. Due to infectious diseases consultation material for mycobacterial culture was obtained. Culture grew M. marinum and the patient was treated with doxycycline for 3 months. Due to incomplete recovery another surgical revision was performed and treatment with ethambutol, rifampicin and clarithromycine for 3 months, followed by clarithromycine monotherapy for another 3 months resulted in complete recovery.
Case 2: A 60 year-old farmer's wife had a little wound due to a raspberry bush. After 4 months she developed a synovialitis of metacarpophalangeal joint. Despite infiltrations with steroids the synovialitis persisted and worsened. 7 months after the initial injury, sugery was performed and the histopathological exam revealed granulomatous inflammation. M. marinum could be identified and a triple therapy with ethambutol, rifampicin and clarithromycine was given for 2 months, followed by 4 months clarithromycine alone.
Case 3: A 21 year-old woman had an injury due to a little wood span. 4 months later she presented with a fistulating wound. The fistula was excised. She was treated with different antibiotics, but the fistula relapsed several times. No osteomyelitis was found. After 10 months, due to an infectious diseases consultation, mycobacterial culture was done and *M. chelonae* could be identified. A treatment with clarithromycine was installed.
These 3 cases illustrate the importance of the awareness of atypical pathogens in relapsing or persisting skin and soft tissue infections. Infections with atypical mycobacteria in non-immunocompromised hosts are rare, but not impossible.

P1813 Identification of mycobacteria by Raman spectroscopy

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Objectives: To analyse the capabilities of Raman spectroscopy for the identification of clinically relevant *Mycobacterium* species.
Methods: Strains were cultured in the MGIT system (Becton Dickinson Microbiology Systems, Cockeysville, Md., US) until indicated as positive by the system. The reproducibility of Raman spectroscopy was evaluated for killed/inactivated mycobacteria versus viable mycobacteria. 16S rRNA sequencing was used as a gold standard for the identification of *M. tuberculosis* complex strains and the most frequently found strains of nontuberculous mycobacteria (NTM). Sixty-three strains, belonging to 8 distinct species were analysed. Biomass from the MGIT system was suspended in 10 microliter of sterile distilled water, transferred onto a quartz slide and allowed to dry. Spectroscopic fingerprints were obtained using a dedicated Raman spectrometer, requiring approx. 1 min. per sample. Cluster analysis on these fingerprints was performed using the pair wise correlations as a distance measure in combination with Ward's cluster algorithm.
Results: The spectra of the inactivated bacteria showed minimal differences as compared to the spectra of viable mycobacteria. Therefore, identification of mycobacteria appears possible without biosafety level III precautions. The reproducibility of the Raman procedure was high. Correlation coefficients between repeated cultures of the same strain were significantly higher than the correlation coefficients between strains of different species. The sensitivity of Raman spectroscopy for the identification of *Mycobacterium* species was 95.2%. All *M. tuberculosis* strains were correctly identified (7/7; 100%) as were 54 of 57 NTM strains (94%). The differentiation between *M. tuberculosis* and NTM was invariably correct for all strains.
Conclusion: The spectra of the inactivated bacteria showed minimal differences as compared to the spectra of viable mycobacteria. Therefore, identification of mycobacteria appears possible without biosafety level III precautions. Raman spectroscopy provides a novel answer to the need for rapid species identification of cultured mycobacteria in a clinical diagnostic setting.

P1814 Characterisation of human mycobacterial isolates using random amplified polymorphic DNA (RAPD) fingerprinting

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Objective: Tuberculosis is a global problem responsible for the deaths 2–3 million people each year. In this study, we developed a

simple random amplified polymorphic DNA (RAPD) assay for rapid identification of different species of mycobacteria and compared with the traditional cultural and biochemical tests.

Methods: We studied 56 clinically isolates and 22 reference mycobacteria strains. The isolates categorised based on growth rate and photoreactive characteristics as nonchromogenic, photochromogen, scotochromogen and rapid grower groups.

Twenty seven commercial primers screened between seven, four, five and six DNA of reference strains of nonchromogenic, photochromogens, scotochromogens and rapid grower mycobacteria by RAPD respectively. The mean of similarity index (Jaccards similarity) of different primers for each clinical isolate compare to reference strain are evaluated. By using sub value 0.65 as a cut off point, maximum genomic similarity of each clinically isolate with related reference strain used for identification of each clinically isolates.

Result: By using short oligomer primers (10-mers) with arbitrarily chosen sequences in the polymerase chain reaction, distinctive and reproducible sets of RAPD profiles were observed for clinically and reference isolates of mycobacteria. The primers 308,304,301 in nonchromogenic mycobacteria, 348,313,AB1–15 in scotochromogens, 348,324,AB1–15,AB1–02 in photochromogens and 348.327,313,301 in rapid grower mycobacteria were identified to be the most suitable primer when tested with related clinical and reference strains. The mean of similarity index of different primers for clinical isolates compare to reference strain of nonchromogenic Mycobacteria group were equal to *M. tuberculosis* in 46 Isolates, Photochromogen mycobacteria group were equal to *M. kansasii* in 2 isolates, Scotochromogen mycobacteria group were equal to M.gordonae in 2 isolates and 1 isolate of each of *M. flavescens* and *M. xenopi*, Rapid grower's mycobacteria group were equal to *M. fortuitum* in 4 isolates and *M. smegmatis* in 1 isolate.

Conclusion: RAPD analysis should be useful in providing genotypic characters for taxonomic descriptions, for typing of Mycobacteria species. The complete procedure required only 48 h from receipt of a mycobacterial culture to final identification and it is a universal system of identifying Mycobacteria to the species level that does not require specialised knowledge and that even allows no specialised microbiologists to make proper identifications.

P1815 Routine use of molecular methods for identification of non-tuberculous mycobacteria species in the clinical laboratory

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Objective: To date, many nontuberculous mycobacteria (NTM) species are isolated from clinical samples. The identification to the species level is frequently relevant for patient management, and molecular methods seem to be the best approach for that purpose. Nowadays, there is not a clear systematic procedure for molecular identification of these microorganisms. The objective of this study was to determine the usefulness and limitations of molecular techniques to identify NTM species and to find the best identification strategy.

Methods: A prospective study of all NTM isolates from infected patients in our geographical area (Costa de Ponent, Barcelona) was carried out (2004–2007; 41-months). Genetic characterisation to species level was determined as follows: a) two reverse-hybridisation commercial systems: INNO-LiPA (June 2004-March 2007) and GenoType assays (since April 2007); b) two in-house methods: PCR-RFLP analysis of hsp65 gene (PRA), and 16S rDNA sequencing (16Seq). In addition, conventional phenotypic methods (growth rate, pigmentation, and biochemical tests) were used in combination with in-house molecular methods.

Results: A total of 489 isolates of MNT were identified during the study period: Four hundred and fifty-one of them (92.2%) were identified by commercial systems: 316 of 347 (91.1%) by INNO-LiPA (12 different species) and 135 of 142 (95.1%) by GenoType (17 different species). The MNT species identified by both methods were as follows: *M. gordonae* (142), *M. avium* (60), *M. chelonae* (44), *M. fortuitum* (44), *M. intracellulare* (40), *M. kansasii* (39), *M. xenopi* (34), *M. avium-M. intracellulare* (17), *M. scrofulaceum* (10), *M. mucogenicum* (4), *M. celatum* (3), *M. genavense* (2), *M. malmoense* (2), *M. marinum* (2), *M. peregrinum* (2), *M. simiae* (2), *M. abscessus* (1), *M. interjectum* (1), *M. smegmatis* (1), and *M. szulgai* (1). The remaining 38 isolates that were not characterised by commercial techniques (7.8%) were identified by PRA (22; 4.5%) or/and 16Seq (16; 3.3%).

Conclusions: Although a high number of different NTM species were isolated in our geographical area, the greater part of them was identified by the available commercial methods. GenoType assay showed a higher level of species discrimination than INNO-LiPA system. Despite PRA and, specially, 16Seq are the finest molecular methods to identify the majority of NTM species, conventional phenotypic techniques should be added in many cases to do a precise and definitive identification.

P1816 Evaluation of multiplex PCR coupled with DNA sequencing of 16S-23S internal transcribed spacer region for rapid differentiation and species-specific identification of non-tuberculous mycobacteria

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Objective: Specific identification is of clinical relevance since treatment varies according to the mycobacterial species causing infection. The aim of this study was to evaluate a multiplex PCR (mPCR) assay for rapid differentiation of MGIT 960 culture isolates as non-tuberculous mycobacteria (NTM) or *Mycobacterium tuberculosis* complex (MTC) members and to confirm the NTM status of each isolate by DNA sequencing of 16S-23S internal transcribed spacer (ITS) region.

Methods: A total of 993 mycobacterial isolates grown with MGIT 960 system over a two-year period in Kuwait were used for evaluation. The DNA from heat-killed liquid cultures was extracted by incorporating removal of PCR inhibitors and mPCR targeting oxyR-ahpC intergenic region and rpoB gene was performed to differentiate NTM from MTC members. The 16S-23S ITS region was amplified and sequenced by using pan-mycobacterial primers. Mixed cultures were identified by a line probe assay.

Results: The mPCR identified 67 isolates (from 49 patients) as NTM, 924 isolates as MTC members and two isolates as mixed cultures. Thirteen different NTM species were identified. Of the 49 individual patient NTM isolates, 11, 10, 6, 5, 5, 2, 2 and 2 isolates were identified as *M. fortuitum*, *M. kansasii*, *M. avium*, *M. intracellulare*, *M. abscessus*, *M. lentiflavum*, *M. gordonae*, and *M. chelonae*, respectively. One isolate each was identified as *M. chimaera*, *M. parascrofulaceum* and *M. immunogenum* while two isolates could only be identified as *Mycobacterium* species. One NTM isolate contained a mixed culture, *M. kansasii* and *M. scrofulaceum*. The repeat NTM isolates recovered from some patients yielded identical results. The 150 randomly selected MTC isolates were identified as *M. tuberculosis* by 16S-23S ITS sequencing and/or hybridisation with *M. tuberculosis*-specific probes. The two mixed cultures identified by mPCR contained *M. tuberculosis* and *M. fortuitum*.

Conclusions: The mPCR accurately and rapidly differentiated all NTM isolates from MTC members. The DNA sequencing of 16S-23S ITS region led to species-specific identification of nearly all NTM isolates. Since majority of mycobacterial infections in developing countries are caused by MTC members, rapid differentiation of all mycobacterial isolates as NTM or MTC members by mPCR followed by species-specific identification of NTM by DNA sequencing is most suitable for proper management of mycobacterial infections.

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P1817 *Mycobacterium avium* ssp. *paratuberculosis* and cytokine response in inflammatory bowel diseases

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Objectives: The studies aimed at analysis of manifestation of *Mycobacterium avium* ssp. *paratuberculosis* (MAP) infections as related

to secretion of selected cytokines (TNF-alpha, IL-1beta, IL-8 and IL-10) in patients with ulcerative colitis (UC) and Crohns disease (CD).
Methods: The studies were performed on three groups of adult patients: group 1 included 20 persons with UC, group 2 consisted of 12 patients with CD, group 3 (control group) was formed by 15 healthy adult volunteers, in whom routine colonoscopy was performed.
Material for the studies consisted of sera originating from the patients of all the three groups and intestinal biopsies from patients of group 1 and group 2. In the sera levels of TNF-alpha, IL-1beta, IL-8 and IL-10 were estimated, employing high sensitivity ELISA kits (Quantikine HS, R and D Systems) for estimation of TNF-alpha, IL-1beta and IL-10 and ELISA kit (Quantikine, R and D Systems) for estimation of IL-8. DNA was isolated from tissue material (QIAamp DNA Mini Kit, Qiagen) and tested for the presence MAP DNA using IS900-specific nested PCR test (Institut Pourquier, France).
Results: In group 1 with UC MAP DNA was detected in 5 (25%) patients. Mean concentrations of studied cytokines amounted to: TNF-alpha – 1.64 pg/ml, IL-1beta – 1.44 pg/ml, IL-8 – 49.74 pg/ml, IL-10 – 2.97 pg/ml.
In group 2 with CD MAP DNA was detected in 8 (66.6%) patients. Man concentrations of studied cytokines were as follows: TNF-alpha – 3.12 pg/ml, IL-1beta – 0.77 pg/ml, IL-8 – 53.70 pg/ml, IL-10 – 2.59 pg/ml.
In statistical analysis MAP DNA was found to manifest significantly more frequently in patients with CD. In parallel, independently of the presence/absence of MAP infection, serum concentrations of TNF-alpha, IL-1beta and IL-8 in patients with inflammatory bowel diseases were elevated as compared to those in the control group while serum levels of IL-10 were the same in all the studied groups.
Conclusion: Inadequate secretion of IL-10 in parallel to the augmented secretion of pro-inflammatory cytokines (TNF-alpha, IL-1beta and IL-8) may play a principal role in development of inflammatory bowel diseases, while the dominating stimulant of pro-inflammatory cytokines production in CD seems to involve MAP.

P1818 Intrinsic drug resistance of *M. avium–M. intracellulare* complex: the role played by efflux pumps

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Objectives: The *Mycobacterium avium–M. intracellulare* complex (MAC) accounts for the nontuberculous mycobacteria most frequently found in clinical specimens, especially in AIDS patients in industrialised countries. Treatment of infections caused by MAC is problematic due to its intrinsic resistance to antibiotics. Although efflux pumps (EPs) have been shown to be of clinical relevance in other bacteria, the importance of drug efflux in mycobacteria is not yet fully understood. In this work, we have characterised the EPs activity in ten MAC clinical strains through an automated fluorometric method and correlated it with intrinsic resistance to antibiotics.
Methods: Two MAC reference strains (*M. avium* ATCC25291T and *M. intracellulare* ATCC13950T) and ten clinical strains isolated from AIDS patients were evaluated for accumulation and efflux of ethidium bromide (EtBr) in the presence or absence of the EPs inhibitors (EPIs) thioridazine (TZ), chlorpromazine (CPZ), verapamil (VP) and the proton uncoupler carbonyl cyanide m-chlorophenylhydrazone (CCCP). For this purpose, an automated real-time fluorometric method that separately assesses accumulation and extrusion of EtBr was used. The effect of the above agents on the MICs of several antibiotics was determined by the broth microdilution method (CLSI).
Results: The automated real-time fluorometric method allowed the detection and quantification of EtBr transport across MAC cell wall. Accumulation of EtBr inside the cell was found to be temperature-dependent and significantly increased by the EPIs TZ, CPZ, CCCP and VP, at half their MICs. The removal of these agents from the medium resulted in energy dependent efflux of EtBr. EPIs promoted the reduction of MICs of MAC strains towards clarithromycin, erythromycin, ethambutol and to a minor extent, rifampicin and amikacin.
Conclusion: The results presented in this work are the first clear evidence of the role played by EPs in the intrinsic resistance of MAC to drugs. In particular, we were able to demonstrate active efflux contribution to intrinsic drug resistance in ten clinical MAC strains isolated from AIDS patients. Furthermore, we found that MAC intrinsic resistance to antibiotics is affected by EPIs such as TZ or CPZ, an effect that might be important in research and development of new and more effective antimycobacterial therapies.

Bacteraemia and endocarditis

P1819 Effect of delay in the administration of adequate antibiotic treatment in *Streptococcus pneumoniae* bacteraemia

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Objective: To determine risk factors associated with mortality in patients admitted to the hospital with *Streptococcus pneumoniae* bacteraemia, particularly analysing the impact of delayed adequate antibiotic administration.
Methods: All adult patients with community-acquired *Streptococcus pneumoniae* bacteraemia admitted to the hospital from January 2005 to November 2007 were enrolled in this protocol study. The following variables were registered: age, gender, source of bacteraemia, severity of illness at hospital admission evaluated by APACHE II score, degree of organ dysfunction (SOFA scale), underlying disease, Charlson comorbidity index, time from the hospital admission to first antibiotic dose, time from the hospital admission to first dose of adequate antibiotic treatment, ICU admission, development of septic shock, and in-hospital mortality. For statistical analysis, categorical variables were evaluated using the chi-square test and Fisher's test when appropriate. Continuous variables were compared using the unpaired Student's t-test and the Mann-Whitney U when appropriate. A multivariate regression model was performed to determine the independent effect of the variables on survival.
Results: One hundred and seventy-five patients were included; 86 of them (71%) were male. The median of Charlson comorbidity index was 6. The source of bacteraemia was pneumonia (n = 144), meningitis (n = 20), others (n = 12). The mortality rate was 19.3% (34 patients). Overall, the median delay of adequate antibiotic treatment was 5 h (15 min – 96 h). In patients with pneumonia, the median delay of adequate antibiotic therapy was 4 h (15 min – 96 h) in survivors and 6.5 h (15 min – 72 h) in non-survivors (p = 0.159). The mortality rate in pneumonia was 25/144 (17.4%) vs 9/32 (28%) (p = NS). In the entire group, the independent risk factors associated with mortality were: age OR 1.05 CI 95% (1.006–1.095), Charlson comorbidity index OR 1.274 (1.029–1.577) and SOFA score 1.319 (1.168–1.490). In patients with *Streptococcus pneumoniae* bacteremic pneumonia age OR 1.067 IC 95% (1.011–1.125); Apache II OR 1.162 (1.014–1.331), time to adequate antibiotic treatment OR 1.122 (1.002–1.255), and septic shock OR 8.27 (1.317–51.94) were the independent predictors of mortality.
Conclusions: After controlling for confounding variables, a delay in the administration of adequate antibiotic treatment is an independent risk factor of mortality in *Streptococcus pneumoniae* bacteraemia of pulmonary source.

P1820 Evolution of *Campylobacter* bacteraemia before and in the era of highly active anti-retroviral therapy

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Objective: *Campylobacter* is a very uncommon cause of bloodstream infection (BSI), but was relatively frequent in HIV positive patients. The impact of HAART and new forms of immunocompromise on CB incidence has not been sufficiently assessed. To analyse the evolution of the incidence, microbiological and clinical characteristics of all cases of CB during a 22-year period.

Methods: Review of the clinical records of all patients with *Campylobacter* bacteraemia (CB) from 1985 to 2007. PreHAART (1985 to 1996) and HAART periods (1997–2007) were compared. Available strains were re-identified with universal PCR.
Results: There were 69 CB in 61 patients (0.25% of all BSI) and the incidence remained stable (mean of 0.06/1000 admissions and 0.49/100,000 inhabitants). Median age was 53 y (IQR 30–74) and 82% were male. Underlying conditions included: liver disease (32.2%), HIV infection (22.7%), neoplasia (12%), solid organ transplantation (SOT)(3.4%) and others (4.7%). CB was community-acquired in 80%. Origin of the BSI was gastrointestinal (34%), unknown (28%) or extra-intestinal (36%:respiratory 15%, intraabdominal 8%, cellulitis 4.5% and urinary 8%). *C. jejuni* was recovered in 68% of the cases (41% primary CB vs secondary 78%; p 0.013), *C. fetus* in 18% and *C. coli* in 10%. Molecular methods reclassified 6 strains: *C. fetus* (2) and *C. jejuni* (1) formerly identified as *Campylobacter* sp and 3 *C. fetus* formerly identified as *C. jejuni*. Complications appeared in 25% of the patients. Quinolone-resistance was found in 67% of the isolates and empirical therapy was appropriate in 64%. Mortality rate was 17% and was higher in HIV + pts (33% vs 10%; p 0.04) and in the presence of complications (50% vs 4%; $p < 0.0001$). Three patients with humoral immunodeficiency recurred (5%).
When preHAART and HAART periods were compared we found that patients with CB were younger in the first period (39 vs 67; $p < 0.001$) and that *C. coli* was only detected in the first period. Although forms of immunocompromise have changed between the two periods, differences did not reach statistical significance: immunocompromise (38% vs 46%; p 0.6), HIV+ (28% vs 15%, p = 0.4), solid (7.5% vs 0%) or haematological neoplasia (2.5%vs 12%), SOT (0 vs 8%).
Conclusions: *Campylobacter* bacteraemia is no longer a disease of HIV+ patients, but often affects immunocompromised patients. CB has an extraintestinal origin in as much as 36% of cases and humoral immunodeficiency must be sought for in patients with recurrent episodes.

P1821 Differences of urinary tract infection patients with and without bacteraemia: a pilot study

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Aim: To assess differences in appearance and outcome of medical patients with documented urinary tract infection (UTI) regarding whether bacteraemia (B) was noted or not
Patients and Method: All medical patients admitted from Emergency of a tertiary hospital hving symptoms compatible with UTI were screened for SIRS criteria [Chest 1992; 1644], prospectively enrolled with all clinical, laboratory and microbiology data entered in PC database (SPSS) until discharge. Only cases with documented UTI were included, and those having B with the same pathogen were matched with an equal number of UTI pts whose blood cultures were negative Analysis by chi square, Mann-Whitney and t-test, as appropriate Time: Feb 2006-November 2007
Results: There were 25 (M: 9) cases of UTI with B (Group A) and 25 (M: 11) without B (Group B) Age and mean N of SIRS criteria on admission for A and B respectively, were 76.8 v 71.7 yrs (NS) and 2.60 ± 0.86 v 1.88 ± 1.01 (p = 0.01) There were similar trends in CRP values, pathogen distribution, comorbidity, previous antimicrobial use or hospitalisation Significant difference was noted in hospital length of stay with a mean of 14.3 ± 11 days for Group A v 7.7 ± 2.8 for Group B (p = 0.01) Mortality tall was higher in bacteraemic patients (7/25) v 2/25 of Group B (p = 0.06)
Conclusions: Patients with UTI eventually proven bacteraemic had a higher sepsis score on admission and special attention should be focused on this simple evaluation tool, as the impact of bacteraemia on prognosis, above all, and also hospital saty was found, as expected, is considerably greater. Seeking microbiology documentation is, obviously, mandatory for patient benefit and prompt management

P1822 Epidemiological and bacteriological profile of community-acquired infective endocarditis

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Background: The epidemiological and bacteriological profiles of infective endocarditis have changed due to longevity, more invasive procedures and immunity disorders.
Objectives: To describe community-acquired infective endocarditis (CA-IE) according to host characteristics and evaluation of the microbiological spectrum.
Methods: Design: prospective study performed in the Cluj-Napoca Teaching Hospital of Infectious Diseases between 1998 and 2007. According to modified Duke criteria 186 definite and probable CA-IE were studied. Bacteriologic assessment was performed using the automatic system BactT/Alert and resistance patterns were determined with API 20E, Api 20NE and ATB automatic methods under CLSI 2006 standards. EPIInfo 6 was used for statistical analysis.
Results: CA-IE was diagnosed in 186 patients (73%) of 254 consecutive cases of infective endocarditis. Most of the cases were native valve endocarditis (153 cases – 82%). Demographic characteristics: age range 7–89 years (median 52 yrs), sex ratio M:F 1.66:1. Immunity disorders were found in 43 cases (23%) including: diabetes mellitus, chronic hepatitis, kidney failure, malignancies, corticosteroid treatment, asplenia. The elderly of more than 60 years represented 30.6% (57) of all cases. Presumed portal of entry represented by poor oral hygiene and periodontal disease, gastrointestinal and urinary tract disorders was identified in 102 patients (55%). The bacteriological profile was dominated by streptococci (20.4% – 38 strains), from which 21% were group C and D. The other common causes were represented by: staphylococci – 29 strains (15.6%), enterococci – 15 isolates (8%) and Gram-negative rods – 8 strains (4.3%). In the elderly the most frequent isolates were streptococci and enterococci. We found significant correlation between digestive or urinary portal of entry and enterococcal aetiology (OR 8; 95% CI, 1.95–32.8) and for periodontal disease and oral streptococci (OR 115; 95% CI, 15.6–2367). Almost all isolates were susceptible to common antibiotics except 10 strains of *Staphylococcus aureus* and coagulase-negative staphylococci that were meticillin resistant. Glycopeptide resistance was never found in these strains.
Conclusion: Underlying diseases and presumed portal of entry are of utmost importance in diagnosing and management of CA-IE. The microbiological profile sustains the usefulness of guideline recommendations.

P1823 Clinical epidemiology and microbiological spectrum of nosocomial endocarditis

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Background: The epidemiological features of infective endocarditis have changed during the last decades because of an increase in placement of prosthetic valves and exposure to invasive procedures.
Objectives: To assess clinical epidemiology and microbiological spectrum of nosocomial endocarditis (NE) defined according to Duke modified criteria.
Methods: Design: prospective study performed in a teaching hospital of infectious diseases.
During the study period (1998–2007) 46 cases of NE were admitted. NE was defined according to accepted definition: endocarditis associated with invasive procedures performed in hospital during the 8 week period before the onset of the disease or endocarditis occurring >48 h after admission.
Results: Definite NE was present in 46 patients (18%) of 254 consecutive cases of infective endocarditis, age range: 21–72 years

(median 49 yrs), sex ratio M:F was 3:1. Comorbities that predispose to infection were found in 14 patients (30.4%): 4 cases – diabetes mellitus, 5 cases – chronic kidney failure, 5 cases – immunosuppressive conditions. Cardiac risk factor represented by prosthetic valves was found in 21 cases – 45.6%. NE was documented based on bacteraemia related to surgical or medical procedures: 22 cases (47.8%) after cardiovascular procedures (less than 1 year after valve placement, intravascular devices, pacemakers implants); 13 cases (28.3%) after genitourinary procedures; 5 cases (10.9%) after gastrointestinal surgical interventions and procedures; 6 cases (13%) after other invasive procedures (endoscopy and ORL procedures). Thirty five percent of NE (16 cases) occurred within the first year after valve implantation (early prosthetic valve endocarditis). The most commonly isolated organism was staphylococci – 21 cases (45.6%), 18 strains *Staphylococcus aureus* and 3 strains coagulase negative staphylococci. Other isolated microorganisms were: enterococci (8 cases – 17.4%), Gram-negative rods (5 cases – 10.9%), uncommon bacteria (2 cases – 4.3%). No vancomycin resistant Gram-positive cocci were isolated. Twenty-three patients (50%) had unfavourable outcome: 4 deaths and 19 complicated cases referred for surgical treatment.

Conclusions: Intracardiac, intravascular devices and genitourinary tract procedures represented the most important risk factors for nosocomial bacteraemia. The leading cause of NE was staphylococci bacteraemia. Cardiothoracic surgery is needed in a high proportion of NE.

P1824 Group G streptococcal bacteraemia and emm types

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Objectives: We studied clinical features and severity of disease in bacteraemia caused by group G beta-haemolytic streptococci (GGS) in relation to emm type of the isolates.

Methods: The medical records of all adult (over 16 years of age) patients in the Pirkanmaa Health District (460 000 inhabitants), Finland, with one or more blood cultures positive for GGS during the 10-year period from January 1995 to December 2004 were retrospectively reviewed. Mortality was recorded within 30 days from the positive blood culture. A severe disease was defined as death during the 30 days or admittance to Intensive Care Unit. Disseminated intravascular coagulation (DIC) was defined as a platelet count lower than 100 x 109 /l. The streptococci were classified for their group antigen G and the isolates were emm sequenced.

Results: GGS grew in 128 cultures; 126 of isolates were analysed, two are underway. 14 different emm types were found; stG480 (27 isolates), stG6 (22 isolates), stG485 (17 isolates), stG643 (10 isolates), stC6979 (9 isolates), stG166b (6 isolates), and stC74a (5 isolates) were the seven most common emm types covering 76% of all isolates. Fifteen isolates remained nontypable (NT). We divided bacteraemia episodes into two groups: those caused by the seven most common emm types (96 episodes, ie. common types) and those caused by less common or NT emm types (30 episodes, ie. rare types). Age or underlying diseases did not differ between the groups. Instead, patients with an ultimately or rapidly fatal underlying disease were more often linked to bacteraemia caused by rare types than that caused by common types [18/30 vs. 36/96; OR 2.5 (95% CI 1.1 to 5.8)]. Mortality was higher in bacteraemia caused by rare types than that caused by common types [10/30 vs. 9/96; OR 4.8 (1.7 to 13.2)]. A severe disease was caused more often by rare types than common types [12/30 vs. 10/96; OR 5.7 (2.0 to 14.2)]. The association between a severe disease and a rare type remained statistically significant also in a multivariate model which was adjusted for the occurrence of an ultimately or rapidly fatal underlying disease and for age [OR 4.8 (1.7 to 13.2)]. DIC was also a more common finding in patients suffering from bacteraemia caused by rare types than common types [5/30 vs. 1/96; OR 19.0 (2.1 to 170.1)].

Conclusion: GGS bacteraemia is caused by variety of emm types, seven of them covering 76% of all isolates. Rare emm types were associated with a more severe disease.

P1825 Bacteraemia complicating pressure ulcers

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Objectives: To evaluate the characteristics and prognostic factors of bacteraemia complicating pressure ulcers (BCPU).

Methods: Prospective study of all episodes of significant BCPU, from 1984 to 2006, in a general hospital. Diagnosis was based on positive blood cultures, isolation of the same microorganism in the ulcer culture, and absence of another source of the bacteraemia.

Results: We studied 47 consecutive episodes of BCPU in 44 patients (17 males, 27 females) aged 77±13 yr. The main causative agents were: *Staphylococcus aureus* (14 cases), *Proteus mirabilis* (13), *Bacteroides* sp. (12), *Escherichia coli* (4), and *Pseudomonas aeruginosa* (4). Bacteraemia was polimicrobial in 11 cases (23%). Dementia, diabetes, stroke and femur fracture were the most common underlying conditions. The ulcers were already present at admission in 41 cases (87%), but the bacteraemia was hospital-acquired in 17 cases (36%). Multiple ulcers were present in 35 cases. The sacrum was the most common site (38 cases) followed by heels (18) and trochanteric region (16). In 5 cases, the ulcers did not show distinct local signs of infection; in 4 of these episodes, *S. aureus* was isolated from blood and ulcer cultures. Surgical debridement was performed in 30 cases (64%). Osteomyelitis was documented in 8 cases. Crude mortality was 42% and mortality related to bacteraemia was 21%. Variables associated with a higher mortality were: septic shock (RR 2.1; 95% CI 1.2–3.7) and serum albumin <23 g/l (RR 2.2; 95% CI 1.1–4.5). Surgical debridement was associated with a lower mortality (RR 0.5; 95% CI 0.2–0.9).

Conclusions: BCPU is associated with a high mortality, although many deaths were not directly related to bacteraemia. Polimicrobial bacteraemia is common. Shock and hypoalbuminaemia were adverse prognostic factors, whereas surgical debridement was associated with a lower mortality. Empirical antibiotic therapy should be active against enteric Gram-negative bacilli, *S. aureus*, and anaerobes including *B. fragilis*.

P1826 Glycopetide-dependent enterococcal bacteraemia following treatment of antibiotic-associated diarrhoea

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Objectives: There are a small number of reports in which Gylcopeptide dependent enterococci (GDE) have been recovered from stool, intra abdominal collections, urine and rarely blood cultures. We describe a case of GDE bacteraemia and its possible risk factors.

Methods: A 21 year old female was admitted with a five day history of pyrexia, abdominal pain and vomiting. A diagnosis of severe necrotising pancreatitis was made and imipenem administered for seven days. Fourteen days after admission the patient developed severe diarrhoea and tested positive for *Clostridium difficile* toxin. Treatment with oral metronidazole and oral vancomycin was initiated and, after a further 48 hours, intravenous vancomycin and gentamicin were also, Vancomycin, intravenous and oral, was continued for sixteen days. On day 30 blood cultures became positive and a Gram stain revealed Gram-positive cocci in chains.

Results: The bottles were subcultured onto blood and chocolate agar. Examination of culture plates revealed no growth on any of the media except around both the vancomycin and teicoplanin discs on the sensitivity plates, suggesting a dependence on glycopeptides for growth. The concentration of glycopeptide required for growth was determined as 0.25 mg/L of vancomycin and 0.094 mg/L of teicoplanin. The organism was identified as *Enterococcus faecium* by DNA sequencing of the 16S rRNA gene. Susceptibility testing performed using IsoSensitest media supplemented with 8 mg/L vancomycin indicated resistance to amoxicillin and rifampicin, but susceptibility to linezolid and quinupristin/dalfopristin. The high level of resistance to vancomycin (MIC > 256 mg/L) and teicoplanin (MIC 32 mg/L) suggested carriage of the vanA gene which was confirmed by PCR.2 The organism reverted

to non-dependence on standard media at a frequency of 2.6×10^{-3} with both dependent and revertant strains having identical genetic fingerprints when examined by pulsed field gel electrophoresis.

Conclusions: We emphasize on association between bacteraemia due to GDE, *Clostridium difficile* associated diarrhoea and prolonged IV and oral vancomycin therapy. As vancomycin at high doses (250 mg, 6 hourly) is now recommended by some authorities for the management of severe *C. difficile* diarrhoea this may facilitate further the problem of glycopeptide dependence in enterococci.

P1827 Infective endocarditis in children: a single cohort study of 51 cases in a French university hospital

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Introduction: Infective endocarditis (IE) in children is rare. Considering the lack of recent data, we performed an epidemiological study to determine diagnostic, therapeutic and outcome features of infective endocarditis in the paediatric setting.

Materials and Methods: We conduct a retrospective study of children IE cases at Necker Enfants-Malades university hospital in Paris (1994–2006). All had a definite IE according to Duke criteria. Data collected included, demographics, underlying heart disease, type of valvular damage, septic localisations, microbiological data, antibiotic therapy and outcome.

Results: From 51 children recorded, 37 (72.5%) were boys, with a median age of 10 years [3 months-17 y]. Median time from symptom's onset to treatment was 7 days. The average follow-up period was 49 months. Thirty nine patients (76%) had congenital heart disease or a valve damaged. Mitral and tricuspid valves were involved in 27% of cases. In 14 cases (27.4%) a foreign cardiac material was infected Thirty two cases (62.7%) had a valvular failure, 3 children had a annular abscess. Ten (19.6%) cases were nosocomially acquired (2 staphylococci, 1 Gram-negative bacillus, 1 enterococcus, 3 yeasts). Portal of entry was identified in 27 cases. Gram-positive cocci accounted for 70.6% (36 cases) (streptococci 17 cases, staphylococci 17 cases and enterococci 2 cases). Twenty patients (39.2%) experienced arterial embolism with lung (10 cases), and brain (6 cases), being the most frequent organs involved. Twelve patients (23.4%) had a severe clinical form (ie, septic shock, acute renal failure, myocardial dysfunction) The treatment was medical alone in 26 cases and medico-surgical in 25 cases.

To date 46 patients (90%) are alive and 5 (9.8%) died, the death occurred in 2 cases during treatment at 8 and 23 days after diagnosis because of ventricular tachychardia. Recurrent endocarditis was detected in 4 children (a new episode of valvular infection occurred in 2 cases and a relapse in 2 cases). One patient surgically treated and 4 treated medically, died

Conclusion: In our children endocarditis study, 20% are nosocomially acquired with foreign cardiac materials in 28%. The most frequent portal of entry was dental, 87% of children had a 4 years survival free of any cardiac event.

P1828 Risk factors for hospital admission in patients with occult *Escherichia coli* bacteraemia discharged from the emergency department

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Background: *Escherichia coli* is the most frequent Gram-negative organism causing bacteraemia. Few studies dealing with occult bacteraemia in adult patients have been published, and none of them addresses specifically *E. coli* bacteraemia.

Methods: In our hospital patients with occult bacteraemia discharged from the emergency department (ED) are followed with a specific protocol. We reviewed all patients with occult *E. coli* bacteraemia discharged from the ED in a ten year period. A total of more than 20 variables including epidemiological and clinical aspects of *E. coli* bacteraemia cases were evaluated. Chi square test or the Fisher test were used for categorical variables and t-test for continuous variables. Logistic regression model was used to identify factors independently associated with hospital admission after detecting bacteraemia.

Results: From January 1997 to December 2006 we identified 261 patients with occult bacteraemia due to *E. coli* discharged from the ED. Of them, 174 (66.7%) required hospital admission. The origin of the bacteraemia was a urinary infection in 207 (79.9%), unknown in 32 (12.4%), biliary in 10 (3.8%) and other origins in 12 (4.6%). A higher proportion of patients who did not require hospitalisation were younger than 65 years (51.1 vs 35.6%, p = 0.03), received adequate antibiotic empirical treatment (84.4% vs 71.3%, p = 0.01) or at least one dose of parenteral antibiotic in the ED (77.6% vs 58.6%, p = 0.001). Among 160 patients who had received adequate antibiotic empirical treatment we detected significant differences in the rates of hospital admission depending on the reception of at least one dose of parenteral antibiotic (62.9% vs 82.9%, p = 0.002). In a regression logistic analysis, age older than 65 years (ORa, 95% CI: 1.74, 1.01–2.99, p = 0.05), and receiving at least one dose of parenteral antibiotic in the ED (ORa, 95% CI: 0.41, 0.23–0.71, p = 0.002) were associated with hospital admission.

Conclusion: Our data suggest that in patients with suspected bacteraemia due to *E. coli* discharged from an ED, initial treatment with at least one dose of adequate parenteral antibiotic could avoid hospital admissions.

P1829 Time-to-positivity of blood cultures in patients with *Klebsiella* spp. bacteraemia

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Background: time to positivity of blood cultures (TTP) is considered a surrogate marker of blood bacterial concentration. We evaluate the relationship between the TTP with clinical parameters, in patients with *Klebsiella* spp. bloodstream infections.

Methods: retrospective study of cases of *Klebsiella* spp. monomicrobial bloodstream infections from January 1997 to June 2007 in our institution. The association of more than 20 variables, including epidemiological and clinical aspects of *Klebsiella* spp. cases, with TTP was evaluated. When multiple cultures were positive only the shortest TTP was selected for the analysis. Chi-square test or the Fisher test were used for categorical variables and t-test for continuous variables.

Results: during the study period we identified 78 cases of *Klebsiella* spp. monomicrobial bacteraemia, with a mortality of 11.5%. Median (interquartile range) TTP was: 12.5 h (10.5–15.1). In one case TTP was unknown. Significant correlation among TTP and the number of positive blood cultures was found (Spearman's coefficient = –0.44, p < 0.001). Association of TTP with several clinical parameters is reflected the table. Mortality in patients with TTP <9 h was 33.3% (2/6), in those with TTP between 9–10 h 14.3% (1/7), and in patients with TTP >10 h mortality was 8.4% (5/64) (chi for trend, p = 0.06).

Characteristics	TTP < 10.5 N = 19	TTP > 10.5 n = 58	p	RR (95% CI)
Nosocomial, n (%)	8 (42.1)	14 (24.6)	0.12	1.79 (0.83–3.83)
Age >65 years, n (%)	15 (78.9)	50 (87.7)	0.28	0.64 (0.26–1.56)
Severe sepsis or shock, n (%)	9 (47.4)	10 (17.5)	0.01	2.7 (1.37–5.6)
Altered consciousness, n (%)	7 (36.8)	6 (10.7)	0.02	2.78 (1.36–5.69)
Required ICU, n (%)	3 (15.8)	0	0.01	
On antibiotic treatment	6 (31.6)	6 (10.9)	0.045	2.39 (1.13–5.02)

Conclusions: in patients with *Klebsiella* spp. bacteraemia, TTP is associated with the severity of the infection and with the outcome.

P1830 Detection of the causative pathogen in infective endocarditis by means of the LightCycler SeptiFAST® test

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Objective: Detection of the causative microorganism in infective endocarditis (IE) in pre-treated patients is still a challenge. At least 35% of cases of culture-negative IE can be attributed to prior antimicrobial therapy.

Reported here is a patient with culture negative IE, in whom the causative agent could be diagnosed by means of the new LightCycler SeptiFAST® test, a real-time based multiplex PCR assay allowing for DNA-detection of 25 pathogens within 6 hours from EDTA-treated blood specimens (Roche, Mannheim, Germany).

Case report: A 58-year-old male patient with malaise and anaemia was admitted to hospital. He reported a history of coronary heart disease and diabetes mellitus type II. Four weeks prior to hospitalisation a transurethral prostatic resection had been performed. Laboratory results showed signs of mild, chronic inflammation. Without definitive diagnosis over a period of 17 days he received amoxicillin/clavulanic acid due to elevated C-reactive-protein levels. Under this therapy he developed fever of 38.6ºC on day 3 after admission. Transthoracic echocardiography showed no pathology at that time and antibiotics were not changed. After 17 days the patient had to be transferred to the ICU because of cerebral deterioration and respiratory insufficiency. The immediate follow-up revealed an aortic valve endocarditis as well as an embolic stroke by means of transoesophageal echocardiography and computed tomography, respectively. Multiple blood cultures remained negative, supposedly because of prior antibiotic therapy. The new LightCycler SeptiFAST® test was applied and revealed *Enterococcus faecalis* (Gram-positive panel, chanel 705, melting temperature 62.6ºC). The treatment was changed to amoxicillin and daptomycin and the aortic valve was replaced. No enterococci could be cultured from valve-tissue, but amplification of part of the 16S rDNA gene (560bp of the V3-region; *Escherichia coli* position 8–575; amplification primers 27F and 556R) followed by cycle sequencing (ABI PRISM Genetic Analyzer 3130; primer 27F) confirmed the presence of *E. faecalis*-DNA.

Conclusion: To our knowledge this is the first report of microbiological diagnosis in IE by means of the LightCycler SeptiFAST® test. Further use of this new molecular-biological tool is warranted to confirm its application in pre-treated IE. The method, however, will not replace conventional blood cultures because no antimicrobial susceptibility data are provided.

P1831 Bacteraemia due to enteropathogenic bacteria in a paediatric hospital in Athens, Greece

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Objective: To review the clinical and bacteriological features of patients with bacteraemia due to enteropathogenic bacteria over a five-year period (2003–2007).

Methods: We retrospectively reviewed the medical records of all included patients for clinical symptoms, laboratory findings, predisposing factors, treatment and outcome. Blood culture, identification, antimicrobial susceptibility and *Salmonella* spp. serotyping were performed by standard methods.

Results: We evaluated 42 bacteremic patients (23 boys, 19 girls). The median age was 18 months (35 days-15 years). Fever (93%) and diarrhoea (76%) were the most common initial symptoms, followed by vomiting (31%) and convulsions (7%). Laboratory findings on admission included: median WBC=11.3×10^9/L (2.9 to 30.6×10^9/L in children with no underlying disease) and median CRP = 76 mg/L (2 to 374 mg/L). Leukocytosis (>10×10^9/L) was present in 23/42 (55%) children and CRP > 10 mg/L in 35/42 (83%). Underlying disease was present in five patients (one with congenital heart disease; three haematogical malignancy and one immunodeficiency/hypoglobulinaemia). The involved pathogens were the following: *Salmonella enterica* (38/42), *Campylobacter* spp. (3/42) and *Aeromonas hydrophila* (1/42). S. Enteritidis was the most predominant serovar (24/38, 63%), followed by S. Typhimurium (3/38, 8%). Nine cases were due to other Salmonellae and only one was due to S. Typhi. Two patients presented osteomyelitis; one immunocompetent (S. Kottbus) and one neutropenic with acute myeloblastic leukaemia (*A. hydrophila*). One child had urinary tract infection due to S. Enteritidis. Overall, 27 patients among 38 tested (71%) had positive stool cultures for the involved bacteria. Twenty out of 38 (53%) patients with *Salmonella* spp. bacteraemia were given third generation cephalosporins; the remaining were given ampicillin or cotrimoxazole. Recurrent disease was not recorded among the evaluated patients. All but one strains were found susceptible to ampicillin and cotrimoxazole, and all strains were found susceptible to cefotaxime and ciprofloxacin. All three strains of *Campylobacter* spp. were susceptible to erythromycin and one was resistant to ciprofloxacin.

Conclusions: Bacteraemia associated with enteropathogenic bacteria is a fairly uncommon entity, but it should be considered in children, especially infants with symptoms of febrile gastroenteritis. Focal symptoms are rare and the usual outcome after proper antimicrobial treatment is favourable.

P1832 The spectrum of invasive bacterial disease in Arctic areas – A population-based study in Greenland

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Objectives: The incidence and mortality of invasive pneumococcal disease in Greenland and in other Arctic areas is high compared with western countries, but it is unknown if this applies only to *S. pneumoniae* or to invasive bacterial diseases (IBD) in general. The objectives of this study were to describe the full spectrum of IBD in Greenland with respect to microorganisms, patient demographics, clinical presentation, severity and outcome.

Methods: For the years 1995–2004 laboratory information of all bacterial isolates from cases of IBD in Greenland were identified. Demographic patient information was obtained from the Civil Registration System in Greenland. Medical information was obtained retrospectively from patient files.

Results: In total 281 unique bacterial isolates from 254 episodes of IBD among 242 patients were identified; 72% of isolates from blood, 18% from cerebrospinal fluid, and 10% from samples from other compartments. In total 20 different bacterial species were identified with *S. pneumoniae* (32.7% of isolates), staphylococci (20.3% of isolates), and *E. coli* (15.7% of isolates) being the most common. The overall incidence of IBD was 45 episodes/100,000/year with highest incidence among children <2 years and the elderly. Incidence was higher in ethnic Greenlanders than in Danes (51 vs. 29 episodes/100,000/year, respectively, age adjusted incidence rate ratio 1.8). Twenty-two percent of patients had an underlying medical condition relevant to the risk of IPD such as chronic lung infection, cancer, systemic immunosuppressive treatment, or HIV. Seventy-six percent of patients smoked and 23% abused alcohol. Overall mortality was 19%; 29% in *S. pneumoniae* cases, while the mortality associated with *N. meningitidis* and *H. influenzae* type B was 0%.

Conclusion: The importance of *S. pneumoniae* as a cause of IBD in Greenland was confirmed, also relative to other IBD-causing bacteria with *S. pneumoniae* comprising 1/3 of isolates, but overall the incidence of IBD appeared not to be uniformly increased compared with western countries. Most IBD cases appeared in persons without known underlying medical risk factors. Prevalence of smoking and alcohol abuse among IBD cases was not substantially different from those of the background population. The known high mortality in Arctic areas associated with *S. pneumoniae* was confirmed, but clinical presentation, severity and outcome of other IBD cases was highly heterogenous and depended on the causal microorganism.

P1833 *Histoplasma capsulatum* var. *capsulatum* endocarditis in metropolitan France

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Objectives: 44 cases of *Histoplasma capsulatum* endocarditis (HcE) have been reported in the literature since 1943. We report 4 additional imported cases of HcE recently observed in metropolitan France through the surveillance performed by the National Reference Center for Mycoses and Antifungals.
Methods: Two HcE cases were collected through the French nationwide prospective study on fungal endocarditis “MYCENDO” (2005–2006). Two additional cases had been observed earlier.
Results: See the table.

Characteristics	Case 1	Case 2	Case 3	Case 4
Year	1993	2003	2005	2006
Age (y)	39	81	58	75
Gender	Female	Male	Female	Male
Stay in endemic area (dates)	Haiti (1954–83)	Guinea, Cote d’Ivoire (1950–4)	Cuba (2002), Brazil (2003)	Brazil (2005)
Predisposing condition	HIV infection	None	Oral prednisone, adalimumab	HIV infection
Prior cardiopathy	No	Mitral valve IE 9 mo before	No	No
Time to Dg	7 d	20 d (autopsy)	1 mo	1 mo
Valve(s) involved	Tricuspid	Mitral	Mitral	Mitral
Other organs involved	Lung, skin, mouth (ulcers)	Lung, mediastinal lymph nodes	Lung	Lung, bone marrow Lung and bone
Mycologic Dg	BAL and bronchial biopsy direct exam. BAL, skin and oral lesions culture; Ab	BAL direct exam.; Autopsy lymph node, mitral valve and lung valve direct exam.	Leg arterial embolism and mitral valve direct exam.	marrow direct exam., PCR (autopsy valve culture medium), Ab
Medical treatment (duration)	AmB (2 mo); oral itra (22 mo)	AmB (1 d)	LAmB (65 d), IV itra (6 d)	LAmB (2 d)
Cardiac surgery (date from Dg)	No	No	Yes (d 1)	No
Outcome (follow-up duration, cause of death)	Death (2 y, *P. aeruginosa* sepsis)	Death (1 d, HcE)	Death (2.5 mo, valve thrombosis)	Death (2 d, HcE)

Ab, antibodies; AmB. amphotericin B; BAL, bronchoalveolar lavage; d, day; Dg, diagnosis; exam., examination; HIV, human immunodeficiency virus; IE, infective endocarditis; itra, itraconazole; IV, intravenous; LAmB, liposomal amphotericin B; mo, months; *P. aeruginosa*, *Pseudomonas aeruginosa*; y, years.

Conclusions: Despite the availability of active antifungals, prognosis of HcE remains very poor and contrasts with that of disseminated histoplasmosis even in immunocompromised hosts; one explanation may be a delayed diagnosis thereby emphasising that European clinicians should suspect HcE in culture-negative endocarditis in patients who stayed in an endemic area, even several years earlier.

P1834 A prospective survey of intravascular-catheter related infection in patients being treated for infective endocarditis

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Objectives: To determine the incidence of intravasular-catheter related infection (CRI) in patients receiving intravenous antibiotics for infective endocarditis (IE) and to examine whether rates of infection vary with type of device used.
Methods: A prospective study of inpatients receiving intravenous (IV) antibiotics for IE over a 26 month period, Jan 1st 2005 – 28th Feb 2007. Patients classified as having Duke possible or definite IE were included in this study. Clinical data was gathered regarding type, site and duration of intravascular catheter used. All cases of CRI (including exit site infections and intravascular catheter-related bloodstream infection, CRBSI) and their sequelae were then recorded.
Results: 103 episodes treated with IV antibiotics for definite or possible endocarditis had complete data sets and were included in the study. 15 (15%) developed CRI. Of these, 12 (12%) developed CRBSI. 5 cases of CRBSI were attributable to a non-tunnelled central venous catheter (CVC), and 7 cases were associated with cuffed, tunnelled venous catheters. None were associated with peripheral venous cannulae. There were 3 cases (3%) of exit site infections in total. 2 cases was related to a cuffed, tunnelled venous catheter and 1 case associated with a peripheral catheter.
CRI were significantly more frequent in patients managed with CVC & tunnelled venous catheters compared to those managed with peripheral cannulae (p =0.0018, Fisher’s exact test). In all cases of CRBSI and exit site infection the culprit device was removed. The most frequently implicated pathogens causing CRBSI were, coagulase negative staphylococci, enterococci and *Candida* spp. There were no serious complications associated with peripheral cannulae.
Conclusion: CRI with serious adverse events occurred during treatment of IE. CRBSI was more common than exit site infections. Rates of CRBSI were greatest with CVC’s and cuffed, tunnelled venous catheters. Peripheral venous catheters and were not associated with CRBSI or serious sequelae. Where possible placement of central venous catheters solely for delivery of antibiotics should be avoided and peripheral cannulae used instead. We are moving away from routine use of central venous access for delivery of antibiotics in these patients due to the frequency and seriousness of complications.

Emerging infectious diseases

P1835 Q fever in New York City: report of a rare and unusual case

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Objectives: Since the year 1999, human Q fever has become a reportable disease throughout most of the United States. It is extremely rare in the New York City (NYC) area, with only 5 cases being reported over the past 6 years. The aim of this study is to describe an unusual Q fever case, which is the first well documented case of its kind occurring in the Queens section of NYC.
Methods: Biopsied tissue samples derived from the patient’s liver and bone marrow were analysed for pathological changes and for pathogenic organisms after staining with hematoxylin-eosin, Giemsa and the Gram stain. Serum was analysed for elevated immunofluorescent antibody (IFA) titers to Coxiella burnetii antigens.
Results: In April 2006, a 46-year-old male resident of Queens, NY, presented with 2 weeks of fever, chills, sweats, and a 1 year history of frequent urination. He appeared anicteric, had undulating fevers with a Tmax of 104oC, but had no rash or cervical adenopathy. He had not traveled outside the NYC area for the past 20 years since immigrating from Guyana, South America, to the USA in 1986. He denied any recent exposure to farm animals or possible insect vectors. CT scan revealed hepatosplenomegaly, and there was a slight pleural effusion on chest x-ray. Laboratory tests revealed leucocytosis, abnormal liver enzyme levels, haematuria and proteinuria. Granulomatous changes appeared in both bone marrow and liver. A non-culturable Gram-negative coccobacillus was identified following bone marrow biopsy. Q fever was suspected and confirmed based on elevated phase I and phase II serum IgM/IgG antibodies against *C. burnetii*. The patient was treated with a 2 week course of doxycycline and his disease status improved dramatically and he has remained symptom-free.
Conclusion: Our findings indicate that an extremely rare case of Q fever occurring in a non-rural or highly urbanised setting emphasizes the need to consider such an unusual zoonotic infection as the possible cause of prolonged fevers, chills, and urinary and/or hepatic abnormalities.

P1836 Outbreak investigation of a Q fever outbreak in a rural setting in the Netherlands

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Objectives: Annually, 5 to 20 cases of Q fever are reported in The Netherlands. In May 2007, an alert was issued in a rural area in the south of The Netherlands about an unusual increase in adult pneumonia cases. These were were later attributed to Coxiella burnetii in what ended up as

the first described human outbreak of community-acquired Q fever in the country. Active case finding and a case control study were performed in the epicentre of the outbreak in order to identify risk factors for acquisition of Coxiella infection and the mode of transmission.
Methods: A case-control study was performed in three villages (population 12500) with the highest attack rate. In total, 35 cases and 697 controls from this cluster area were invited to participate in the study to achieve at least a 1:2 case-non case ratio, taking expected seropositivity rates (15%) and anticipated participation rates into account. All study participants were screened for acute Q fever infection with IFA (Focus Diagnostics) for phase I and II IgG and IgM antibodies with a single 1:64 serum dilution. A standardised questionnaire was administered for identification of potential risk factors. Univariate and multiple logistic regression analyses were performed. Geospatial analyses were done to assess the distance of cases' residence to goat farms in the area. Environmental and animal samples were taken from a commercial and a hobby goat farm.
Results: Of the 408 participants at risk at the beginning of the study, 76 (18.6%) were found to be positive for acute Q fever infection. The most frequent symptoms were fatigue (44.0%), headache (43.1%), influenza-like symptoms (41.3%), cough (37.0%) and fever (30.6%). The overall attack rate in the most affected village was 12.6% (age range 18–84 years, corrected for overall age and sex distribution). Both farms were positive for *C. burnetii* by use of PCR (41 out of a total of 77 samples). Preliminary risk analyses showed that attack rates for acute Q fever infection were higher in the proximity of positive goat farms. The extremely dry weather conditions in April may have played a contributing role in this outbreak.
Conclusion: Over 260 inhabitants of the most affected village are estimated to have acquired *C. burnetii* infection in the spring of 2007. *C. burnetii* infected goat farms in the region appear to be a risk factor. Further analyses should elucidate if other risk factors were involved as well.

P1837 Pulmonary involvement in leptospirosis, Northern Greece

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Objectives: Leptospirosis is a zoonotic disease of great importance, caused by spirochetes of the genus Leptospira. Its clinical presentation may vary from a flu-like-syndrome to the severe form of Weil's disease. Recently, co-existing pulmonary manifestations are becoming more prevalent, with a reported incidence of 20–70%. Leptospirosis is endemic in Greece, and cases, sometimes severe or even fatal, occur every year. Aim of the present study was to find out the prevalence and the severity of respiratory leptospirosis cases in Northern Greece.
Methods: Epidemiological and clinical data from 123 patients with confirmed leptospirosis who were hospitalised during 1998–2007 were analysed retrospectively. The median age of patients was 51 years (6–83 years) and most (82.1%) of them were male. Diagnosis of leptospirosis had been achieved by serological and/or molecular methods.
Results: The source of infection was defined in 63 cases: 73% rural, occupational exposure (farmers), 14.3% urban occupational exposure, 6.5% house-holding activities, 3.2% recreational activities. The icteric form of the disease was present in 44.7% of the cases, and 22% had the classical Weil's disease. The mortality rate was 8%. Among patients, 25.2% presented pulmonary manifestations, with main symptoms being cough, haemoptysis and dyspnea; half of those cases turned out to Adult Respiratory Distress Syndrome or Acute Respiratory Failure. The mortality rate of patients with respiratory involvement was 28.6%. It was found that pulmonary manifestations in leptospirosis correlate significantly with the mortality rate (OR = 5.2, 95% CI: 1.38–20.18, $p = 0.015 < 0.05$).
Conclusion: Leptospirosis in Greece is closely related to rural occupation and affects mainly males. Jaundice is a very common symptom, most probably related with the causative Leptospira species. Respiratory leptospirosis is quite common (25.2% of hospitalised cases) and appears to be a high risk factor for fatal outcome, deteriorating the patient's prognosis and increasing mortality rate. Prompt and accurate diagnosis and treatment are essential for patients' life.

P1838 Leptospirosis: prognostic factors associated with mortality

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Background: Leptospirosis is a zoonosis that occurs worldwide. It is characterised by great clinical variability ranging from a mild flu-like illness to acute life-threatening condition. We conducted a retrospective study to identify the factors associated with the prognosis and mortality of this infection.
Methods: All the episodes of leptospirosis diagnosed in the Hospital Central de Asturias, a universitary hospital in the Northern of Spain, between 1997–2007 were retrospectively reviewed. The diagnosis was based on a positive serology using an indirect agglutination test or IgM specific ELISA and a clinical setting consistent with leptospirosis. Continuous valous were compared using Student t test or U of Man-Whitney. Categorical values were compared using Fisher's exact test or chi2-test. A p value less than to 0.05 was considered statistically significant
Results: Twenty-three episodes were reviewed. Seventy-three (70%) patients were male, (mean age 51 years, limits 17–79). All patients have pertinent epidemiological context, including occupational exposure (19 cases) or swimming in a river (4 cases). All patients have positive agglutination from 1/32 to 1/10565 and fourteen patients have IgM positive. The mean delay between onset the first symptoms and hospital admission was 6±4 days (limits 2–18 days). In the blood test all the patients showed impaired liver function: AST (mean 465 IU/L; limits 100–4.300), ALT (mean 678 IU/L; limits 111–8.862) and bilirrubine (mean 11.68; limits 4–40). Nineteen patients showed impaired renal function: creatinine (mean: 3.6; (limits 1.2–7.6) and urea nitrogen (mean 128, limits 33–267). All patients received antibiotic treatment except one patient that dead before the treatment. The treatments were: intravenous penicillin (8 cases), ampicillin (6 cases), third-generation cefhalosporines (6 cases) and doxicycline (one case). 30% of the patients were admitted to the ICU. The most frequents complications were: distress respiratory (9 cases), haemorrhages (4 cases) and renal insufficiency (5 cases). Seven patients (30.6%) dead due to infection. The mortality was associated with: impaired renal function (P = 0.20) and presence of distress respiratory (6 vs 9, p = 0.007, OR = 0.04) in univariable and multivariable analysis.
Conclusion: The mortality of leptospirosis remains high despite the treatments and admission in intensive care unit. The mortality is associated a severe renal disease, and lung complications.

P1839 Leptospirosis: overview of the 2004–2007 cases in a Portuguese population

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Leptospirosis is a very acute disease caused by Leptospira interrogans (sensu lato). The range of symptoms can vary from flue like symptoms to renal failure, haemorrhagy and jaundice. The disease is widely spread all over the world, with a higher incidence is the tropical areas of the globe. In Portugal, the average number of declared cases for the past 5 years (2002–2006) was 48 cases/year, with a higher incidence on adult men. The Instituto Nacional de Saúde Dr. Ricardo Jorge (INSA) is one of the few laboratories in the country diagnosing the disease, and receives serum samples from all over Portugal to perform the MAT (Microscopic Agglutination Test).
The main objective of this work is to present an overview of the main results obtained for the leptospirosis diagnosis in INSA.
Mat was performed on 1640 serum samples sent to INSA by hospitals from all the country, during 2004–2007. The tested serogroups were: Australis, Autumnalis, Canicola, Celledoni, Cynopteri, Grippotiphosa, Icterohaemorragiae, Louisiana, Mini, Pomona, Rachmati, Sejroe, Shermani. These samples came from patients who were suspicious for

leptospirosis. 98 patients were positive (presented MAT titers ⩾100). 75% of the positive cases were male. The average age was F=55 years (min = 18 max=75); M=57 years (min = 23 max=86). These results are consistent with the epidemiology of the disease, being a disease of rural workers. Cases occurred mainly in the spring and autumn, the rainiest seasons in Portugal, as expected. In fact, the spread of leptospires is increased during the wet seasons. The majority of the cases (52%) occurred in the North of Portugal (more rural areas), 23% in the Lisbon area. The analyses of In the north of Portugal, the most frequent specific serogroup agglutination found was with Louisiana (n = 9; 17% of the cases), Pomona (n = 7, 14%) and Ballum (n = 5, 10%). In the Lisbon area, the most frequent specific serogroup agglutination was Ballum (n = 5, 23%) and Australis (n = 4, 17%). In 24% of the cases no specific serogroup agglutination was observed. In these cases, sera presented mild agglutination in most of the serogroups tested.
Leptospirosis is still an important health problem, with a considerable number of cases/year in Portugal, and, in the eminence of climate changes, it may become a very important problem in Mediterranean countries.

P1840 Influence of climatic factors on tick-borne encephalitis incidence in the Czech Republic

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Objectives: The major objective was to find out whether there exist any demonstrable relationships between the incidence of human tick-borne encephalitis (TBE) infections and meteorological factors, to specify their character and make an attempt at an interpretation of the action of the latter.
Methods: Epidemiological data were extracted from the EPIDAT database (National Institute of Public Health, Prague). The basic datum characterising each case was the date of primary symptoms, whereby in comparisons with the course of the weather enabling to respect the incubation period (since the attachment of an infected tick). Analysed was a total of 4613 cases of disease in 1994–2001. The results obtained were compared with those in later years.
Meteorological data were from the database of the Czech Hydrometeorological Institute in Prague. That contained data (9 parameters) from 22 selected stations located in a region of the Czech Republic known for its continued incidence of TBE.
Statistical methods testing the relationship between TBE occurrence as a dependent variable with meteorological factors in the role of predictor, were carried out with the aid of the STATISTICA6 package.
Results: A linear relationship has been found between TBE incidence and temperature factors in all the years under study. Quadratic relations (with a shift in time by 6–15 days with respect to incubation of the infection) were demonstrably better in precipitation, relative humidity of the air and soil. In the period under follow-up, vast year-to-year differences in weather characteristics were observed over the warm period. In combination with data on TBE incidence it was possible in each year under follow-up to identify an extraordinary in character period of time fundamentally influencing this infection.
Conclusions: Effects of the current course of the meteorological situation (as well as long-term year-to-year changes) on TBE incidence are foremost mediated by the influence of climatic factors on *Ixodes ricinus* ticks and their host-seeking activity. These mutual connections have been demonstrated in parallel a long-term monitoring of this vector and the manifested human TBE virus infections. Under certain conditions there also have to be taken into account the effects the momentary meteorological situation has on human behaviour in as TBE in the Czech Republic is a recreational infection.

P1841 Shifts in the altitudinal distribution of the tick-borne encephalitis virus and the incidence of disease influenced by the warming climate in the Czech Republic

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Objectives: The sharp increase in tick-borne encephalitis (TBE) incidence in the 1990s and its high level were not evenly spread over the whole territory of the Czech Republic. It was manifest markedly in the Highland Region where TBE incidence is double that of the countrywide average. Therefore, an environmental epidemiological study has been undertaken and the results compared with a long-term follow-up of changes in the vertical distribution of *Ixodes ricinus* ticks and of the infectious agents transmitted by them, as influenced by climate warming in the area of the Krkonoše Mts., the highest mountain range in the Czech Republic.
Methods: Data on the incidence of human cases of TBE, including the places of probable infection have been excerpted from the EPIDAT data base (National Institute of Public Health, Prague). Meteorological data are from the data base of the Czech Hydrometeorological Institute (CHMI), Hradec Králové and from CHMI, Prague. Data on cleft-hoof game are from the data base of the Czech Ministry of Agriculture. For the evaluation of the average annual and monthly temperatures, the least-squares multiple linear regression was used.
Results: In the decades 1971 through 1992 the incidence of TBE in the Highland Region didn't reach the countrywide average, a rise being noted only since 1997; in the year 2006, TBE incidence was more than double that of the countrywide average. There have not been found any socio-economic or land-use changes or any in the numbers of game animals, that could have had an effect on the situation. The rise was markedly steeper in localities with occurring infection above 500 m a.s.l. than below that level. At those altitudes there was observed an increase in average monthly temperatures above the countrywide average, namely over the period of maximum *I. ricinus* activity (May–August). Comparisons with results of long-term field experiments in the Krkonoše Mts., confirm the causal connection between the meteorological phenomenon and increased TBE incidence.
Conclusions: Analyses made show that in locations at higher altitudes (at the previously observed vertical limit of *I. ricinus*) the increase in average temperatures in the spring-summer season is greater than that of the overall average in the Czech Republic. At a summer vertical gradient of 0.6 ºC/100 m, a systematic 1.1–2.8ºC change in the Czech-Moravian Highland corresponds to environmental conditions formerly prevailing at altitudes lower by around 200–450 m.

P1842 Prevalence of antibodies to *Bartonella henselae* in patients with suspected cat scratch disease in Crete, Greece

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Introduction: Cat scratch disease (CSD) is caused by a fastidious intracellular Gram-negative bacillus as result of cat contact or kitten fleas contact. In immuno-competent patients it causes a self-limited disease, which often resolves without therapy, characterised mainly by fever and regional lymphadenopathy. In immuno-compromised patients it causes bacillary angiomatosis, liver and splenic peliosis.
A number of atypical cases of CSD implicating encephalitis, endocarditis, osteomyelitis, Parinaud occuloglandular syndrome, granulomatous hepatitis, neuroretinitis, transverse myelitis, show severe morbidity and complicated course. Symptoms such as persistent fever, night sweats and malaise can simulate malignancy leading to expensive, unnecessary and often invasive evaluations.
Patients and Methods: From January 2006 to November 2007 470 serum samples, of patients hospitalised in the 5 major hospitals in Crete, were examined using IFA. Titers of IgG ⩾ 1/512 or IgM ⩾ 1/80 or IgG ⩾ 128 and IgM ⩾ 40 were considered as serologically positive.

Results: Of the 470 samples tested, 15.7% (74/470) revealed positive IgG titers, 19.1% (90/470) revealed positive IgM titers and 21.7% (102/470) revealed positive titers for both IgM and IgG. Thirty seven percent (65/176) of males and 36.5% of females (95/260) were serologically positive. Of the 57 patients for which a second sample was, also, tested, 34 (59.6%) showed seroconversion. During the 4 seasons the seropositivities were: December-February 26.9% (28/104), March-May 35% (43/123), June-August 39.3% (48/122) and September-November 33.3% (48/144).
Of 40 of the seropositive patients, medical and epidemiological history was obtained and the predominant clinical manifestations were fever (60%), lympadenopathy (67.5%), spleen enlargement (10%), arthralgia (7.5%), headache (5%), malaise (5%), anorhexia (5%), vomits (2.5%), haematuria (5%), rash (5%), impaired vision (5%), tonsilar hypertrophy (2.5%) and red swollen tonque (2.5%). Other symptoms were: rhinitis, Gullain Barrett syndrome, encephalitis, pericarditis and liver enlargement. Cat scratches or contact with cats were confirmed in 10% of cases.
Conclusions: Accurate and timely diagnosis is, therefore, of clinical importance.

P1843 Seropevalence study of cat scratch disease in Crete, Greece

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CSD is considered as an emerging disease and its causative factor has been established to be *Bartonella henselae*. The primary reservoir of this bacterium is the cat and its fleas.
Purpose: The purpose of this study was to establish the seroprevalence of *B. henselae* antibodies in a representative sample of healthy blood donors in the island of Crete.
Materials and Methods: 481 samples of healthy blood donors (statistically representative sample) were collected randomly, from July 2005 to October 2006, from the respected units of the major hospitals in the 4 prefectures of Crete. All samples were tested by IFA (FOCUS, USA) against IgM and IgG antibodies of *B. henselae* (cut-off points: IgG $\geqslant$1/64, IgM $\geqslant$1/20). To perform the statistical analysis the donors were separated into 5 age groups (<20, 21–30, 31–40, 41–50, >51), gender (males and females), 2 groups based on occupation (low and high contact possibility with the pathogen), and 2 groups based on residence (urban and rural). The statistical analysis was performed using SPSS v. 13.
Results: The seroprevalence against IgG antibodies was estimated at 43.2% (208/481), while the titers ranged from 0 to 1/1024. Seventeen percent of these IgG positive sera, also, revealed positive titers against IgM antibodies.
In each respecting prefecture, the seroprevalence was as follows: Prefecture of Heraklion: 48.1% (78/162), prefecture of Rethymnon: 45.2% (42/93), prefecture of Chania: 50.5% (52/103), prefecture of Lassithi: 34.1% (31/91). A lower seroprevalence was recorded in males (42.2%) than in females (44.9%). The highest seroprevalences were observed among the age groups of 41–50 (49.2%) and 21–30 (43.9%). No statistically significant differences were revealed among the age groups (t = 0.81, f = 0.39), gender (t = 0.39, f = 0.71), and residence (t = 0.99, f = 0.00). A statistically significant difference was observed between the two occupation groups (t = 0.038, f = 4.35).
Conclusions: We confirmed the increasing frequency of the Cretan population towards the exposure to the pathogen. Due to the high seroprevalence revealed, it is guessed there might be inconspicuous *B. henselae* infections among the population. Clinicians should include *B. henselae* as an agent of potential infection in patients with lymphadenopathy, fever, and history of cat contact.

P1844 Changes in the activated cytotoxic suppressor T-lymphocytes in elderly patients with Mediterranean spotted fever

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Aging of the population is the process with the steadiest development. Clinical observations of the aging process lead to the conclusion that old age is the most widely distributed type of immunodeficiency.
Objectives: To find if changes happen in the cytotoxic suppressor T-lymphocytes in elderly patients with Mediterranean spotted fever(MSF).
Methods: We investigated the cell immunity of 20 patients of age over 60 years with MSF, using immunofluocytometry. The control group was composed of 10 young patients and 10 healthy individuals over the age of sixty. The data were processed using several statistical methods. In the investigated group 43.3% of the patients had severe form of the disease, 46.6% had intermediate clinical course, 10.1% had mild forms.
Results: Significantly more increased expression of CD8+/CD38+ was found in the elderly patients (22.35±4.71), compared to the patients of the control group – (22.35±5.76) for the young patients and (22.43±7.31) for the healthy persons over 60 years ($p<0.05$ for both). The correlative dependence r for CD8+/CD38+ for the healthy elderly is 0.59; for the young patients is 0.37; for the elderly in the investigated group is 0.03. These results are determined not only by the age but also by the severity of the illness.
Conclusion: Expression of the activated cytotoxic suppressor T-lymphocytes in elderly patients with MSF is increased and shows correlation with the severity of the disease.

P1845 Tick-borne rickettsiosis in the Altai region of Russia

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Objectives: North Asian tick typhus (NATT) is a tick-borne zoonosis caused by *Rickettsia sibirica* ssp. *sibirica* (*R. sibirica*) in the Asiatic part of Russia. More than 66,000 cases of NATT have been registered in Russia from 1936 to summer 2007. In the Altai region of West Siberia, the average morbidity is 50 100,000 inhabitants per year. NATT is transmitted to humans by *Dermacentor nuttalli*, *D. silvarum*, *D. marginatus*, *D. reticulatus*, *Haemaphysalis concinna* and *Ixodes persulcatus*. In addition, *R. sibirica* and *R. raoultii* were detected using PCR in *D. nuttalli* ticks collected in the Altai Republic. We carried out molecular identification of a collection of 15 spotted fever group (SFG) rickettsiae cultivated from patients and from ticks from Altai from 1965 to 1989. We also screened ticks collected in forest-steppe zone of Altai region (Aleiskiy, Byiskiy and Shypunovskiy districts) for alpha-protobacteria from the order Rickettsiales.
Methods: Rickettsial strains were identified using partial amplification of the 5′-end of the ompA gene (190–70 and 190–701 primers). alpha-protobacteria were detected in ticks by amplifying the gltA gene (CS1d-CS535r and CS409d-RP1258n primer pairs). Positive PCR products were sequenced and compared to GenBank.
Results: Fourteen strains were identified as *R. sibirica*. These strains were isolated from humans (2) and six ticks species (*D. nuttalli* [2], *D. silvarum* [2], *D. marginatus* [4], *D. reticulatus* [2], *H. concinna* [1] and *I. persulcatus* [1]). The remaining strain, isolated from *H. concinna*, was identified as *R. heilongjiangensis*.
In *D. reticulatus* and *D. marginatus* ticks, we detected *R. raoultii*. In *I. persulcatus* collected in Aleiskiy district, we detected “Candidatus *R. tarasevichiae*”, *Ehrlichia muris*, *Anaplasma phagocytophilum*, a rickettsia closely related to *R. tamurae*, bacterium “Montezuma” and *R. raoultii*. Several of these ticks were co-infected by two or more of these bacteria. In *H. concinna*, we detected a co-infection with *R. heilongjiangensis* and *R. raoultii*.
Conclusion: We confirm the aetiological role *R. sibirica* as agent of NATT, and the role of six tick species as vectors for this agent. However, we detected several other members of the order Rickettsiales, some

of which being recognised pathogens, in ticks from Altai. Our results highlight the need to pursue molecular identification of tick-borne human infections in this region.

P1846 Diphtheria in Latvia – Still important healthcare problem

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Introduction: Diphtheria in Latvia and other former USSR republics broke out during the last decade of the 20th century. The reason was shortcomings in diphtheria surveillance and immunisation programmes. The highest number of cases in Latvia was recorded in year. The aim defined by World Health Organisation in 1999 was to decrease diphtheria incidence in Europe by year 2010 to 0.1 cases per 100,000.
Aims, Material and Methods: Using retrospective analysis of case-records – to summarize results of immunological examinations, peculiarities of the course and outcomes of diphtheria in 25 patients treated in Infectology Center of Latvia (ICL) during the year 2006. The other aim was to detect level of antitoxic antibodies in blood of ICL employees by *Corynebacterium diphtheriae* IgG-ELISA (NovaTec immundiagnostica GmbH).
Results: There are 32 diphtheria patients registered in Latvia during the year 2006, it means – 1.4 per 100,000 inhabitants. Twenty five of them were treated in ICL. Patients age ranged from 18 to 63 years. In all cases *Corynebacterium diphtheriae* toxig. gravis was isolated from pharynx. From those only 2 patients in the age group between 20 and 25 years were vaccinated. Others either were not vaccinated according to defined scheme or considered themselves non-vaccinated. In ten patients severe course of disease with complications – myocarditis, nephritis, bulbar syndrome and polyneuropathy developed. There were 3 lethal cases. Mild course of disease was observed in two vaccinated 20 and 23 year old subjects. The level of determined antitoxic antibodies indicated that patients have not good individual protection from diphtheria.
Antitoxic antibodies against *Corynebacterium diphtheriae* were detected in 340 employees of ICL. Vaccination according to the defined immunisation scheme has been performed only in 209 persons. In 70 persons concentration of antibodies were below protective level of 0.1 IU/ml. Twenty two of them were vaccinated according to the defined scheme. The lowest level was observed in employees older than 45 years.
Conclusions:

1. The high incidence of diphtheria in Latvia is due to lack of protective immunity.
2. Priority in prevention of diphtheria is vaccination of all non-immune individuals.

P1847 *Weissella* spp.: lactic acid bacteria emerging as a human pathogen

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Objectives: *Weissella* is lactic-acid bacteria closely related to *Leuconostoc* and *Lactobacillus*. Human infections are rarely reported. We reviewed *Weissella* clinical isolates in our institute during the past decade.
Methods: Catalase-negative, Gram-positive bacteria from clinical specimens from 1997-present were studied. The bacteria were tested by manual phenotypic assays (vancomycin MIC, Gram's stain, PYR, LAP, gas from MRS broth, and ADH), API 20 STREP commercial assay, and some by API 50 CHL. Isolates phenotypically identified as *Leuconostoc*, *Lactobacillus* or *Weissella* were subjected to 16S rDNA sequencing (J Clin Microbiol 2003;41:4134). Final identification as *Weissella* required both compatible phenotypic characteristics and at least 99.5% sequencing similarity to a *Weissella* GenBank isolate plus a significantly lower degree of sequence matching with the other genera. Clinical details of the patients were then studied for the *Weissella* isolates.
Results: Of thousands of catalase-negative coccoid or coccobacillary bacteria, 51 met either the manual or the commercial phenotypic criteria. 11 isolates had final identification as *Weissella* (Table). A young female with arteriovenous malformation involving left mandible, having undergone surgical removal and bone grafting, suffered from postoperative osteomyelitis with chronic discharge. She received ampicillin-sulbactam and operative debridement of necrotic bone. Culture of the bone tissue grew *Weissella confusa*. An elderly female with long-standing bilateral uveitis and reactive VDRL and TPHA underwent lumbar puncture to evaluate for neurosyphilis. The cerebrospinal fluid had visible blood contamination, revealed no evidence of inflammation nor reactive for VDRL, but grew *W. cibaria*. She received penicillin therapy for syphilis (which might as well have covered *Weissella*). All positive blood cultures were of either single-bottled or one-of-two types. Although weissella is not generally considered skin flora, transient 'bacteraemia' in our cases makes clinical correlation difficult.
Conclusion: *Weissella* has been rarely reported clinically. The organism is of low virulence. Infections occur mostly in patients with impaired host defence, with foreign materials, and with antimicrobial pressure. Without knowledge of this group of organisms and availability of full-scale phenotypic and genotypic assays, these bacteria would be identified as either *viridans* or other streptococci, *Leuconostoc*, or *Lactobacillus*.

Organism % seq. identity	Clinical specimen	Relevant clinical diagnosis	C* or H*	Host status	Antibiotic pressure	Foreign body or device	Clinical significance	Treatment & outcome
W. cibaria 100%	blood	chronic arthritis	U	normal	yes	tiny piece of wood	probable	drainage & antibiotic recovered
W. confusa 99%	blood	fever with rash	C	Infant	no	no	probable	spontaneous recovery
W. viridescens 99.9%	blood	+ve blood culture	U	diabetes stroke	no	PEG tube	possible	antibiotic expired
W. cibaria 100%	postmortem lung swab	probable aspiration pneumonia	C	epilepsy	no	no	probable	expired on arrival
W. confusa 99.6%	bone	chronic osteomyelitis	H	postoperative complication	yes	no	yes	debridement antibiotic recovered
W. cibaria 99.5%	urine	sepsis	C	multiple myeloma	no	no	unknown	N/A
W. confusa 99.9%	blood	bacteraemia	H	alcoholic liver dis. stroke	yes	aneurysm clips	unknown	N/A
W. confusa 99.6%	blood	pneumonia	C	biliary atresia	no	no	probable	antibiotic recovered
W. cibaria 99.5%	CSF[†]	uveitis	C	chronic uveitis latent syphilis	no	no	unknown	N/A
W. confusa 99.6%	blood	fever	H	bile duct carcinoma	yes	yes	probable	antibiotic recovered
W. confusa 100%	ND	ND	ND	ND	ND	ND	ND	ND

*community or hospital acquired; U = uncertain; N/A = not applicable; ND = no data.

P1848 Outbreak of acute gastroenteritis caused by Staphylococcal enterotoxins affecting an Austrian school centre

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In September 2006 an outbreak of food poisoning (FP) in a federal accommodation facility for pupils in Eisenstadt (Burgenland) in Eastern Austria was reported. Shortly after a lunch that was considered responsible for this FP, 113 (of 240) persons fulfilled the outbreak case definition; yielding an overall attack rate of 47.1%. Eighty individuals (70.8%) suffered from diarrhoea, 77 (68.1%) from abdominal cramps, 103 (91.2%) from nausea and vomiting, 28 (24.8%) developed fever and 60 (53%) had symptoms of hypotension. Most of these pupils required fluid replacement and a total of 101 persons were hospitalised. All 113 patients recovered within a period of no more than 48 h.
Previous investigations, which excluded *Salmonella*, EH *E. coli*, *Yersinia*, *Campylobacter* or norvovirus as causative pathogens, pointed towards *S. aureus* related FP. In order to identify the bacterial toxin pattern, clonality and source of contamination, we conducted bacterial investigations on *S. aureus* recovered from stool specimens of patients, the suspicious meal as well as from nasal and palmar swabs from food handlers.
Materials and Methods: *S. aureus* isolates were analysed for clonal similarities by pulse-field gel electropheresis (PFGE). To define the

individual toxin-pattern of each bacterial isolate, screening of 19 previously described staphylococcal pyrogenic toxin superantigen genes was performed by single gene PCR. The toxins investigated included the toxic shock syndrome 1 gene tst, and staphylococcal enterotoxin- (se) and enterotoxin-like (sel) genes such as sea to see, seg to selr and selu.
Results: *S. aureus* was isolated from the rice meal, from nasal and palmar swabs of the foodhandlers and from 44 stool samples of the patients. Almost all examined bacterial isolates (41 out of 48) displayed an identical PFGE-pattern (1 meal, 1 palmar, 39 stool) thus indicating a clonal outbreak. Moreover, an identical staphylococcal PTSAg gene pattern (sea, sed, selj, selr) could be identified in 39 bacterial isolates (1 meal, 1 palmar, 37 stool) by PCR.
Conclusion: We hereby confirmed the clonality and identified the enterotoxin pattern associated with a recent outbreak of *S. aureus* food poisoning in Austria. Moreover, our results enabled us to track down the source of *S. aureus* to a food handler. These results, together with the clinical course of food poisoning, once again underline the importance of personal and kitchen hygiene among food handlers.

P1849 A case of pneumonia caused by *Bacillus anthracis* secondary to gastro-intestinal anthrax

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Objective: Gastrointestinal anthrax (GIA) is a very rare form of anthrax with high fatality rate. We report here a fatal case of pneumonia due to *Bacillus anthracis* secondary to GIA.
Case: A 59 years-old man with carotid stenosis, diabetes mellitus and hypertension was admitted to our hospital for performing of the elective endovascular carotid stent procedure. He had only a five-month history of vision loss. On physical examination; his general status was good with normal vital signs including body temperature (36.7ºC). Systemic findings were normal except vision loss. During the endovascular procedure, suddenly he complained severe abdominal pain. His general status became bad with tachycardia (110/min), tachypnea (32/min) and hypertansion (240/140 mmHg). He was transferred to the intensive care unit (ICU). Laboratory findings were as follows; WBC count of 11400 /mm^3, ESR 32 mm/h, CRP 2.33 mg/dl (Normal: 0–0.8). On the following day, acute abdomen was developed and laparotomy was performed suspicion of mesenteric ischaemia. Resected bowel section was sent to patology. Ceftriaxon (1g every 12 h) and metronidazole (500 mg every 8 h) therapy was started. His general status and vital signs were normal after the surgery. On the postoperative second day, he became febrile (39ºC) and respiratory arrest was developed. After the entubation; cultues of tracheal aspirate and blood were performed. Following day, non-haemolytic Gram-positive bacilli similar to *Bacillus anthracis* yielded on the culture of the tracheal aspiration material. It was found out that he had consumed meat from endemic region for anthrax from his detailed history. Previous antibiotic therapy was stopped. Crystalise penicillin G (4MU every 4 h, intravenously) and ciprofloxacin (200 mg every 12 h, intravenously) was started with the diagnosis of anthrax pneumonia. The capsule formation of the bacilli was detected after 5 hours incubation of the bacteria in the defibrinated sheep blood. *B. anthracis* isolated from the patient was susceptiple to penicillin by disk diffusion testing. Encapsulated Gram-positive bacilli were seen in the Giemsa and Gram stain of the paraffin tissue section from the necrotic ileum parts. His fever persisted and unfortunately he died in spite of the all supportive therapy on the nineth day of the treatment.
Conclusion: Anthrax should be considered when suspected bacilli obtained from any culture of the patients especially in endemic countries.

P1850 A rare case of *Nocardia puris* postoperative endophthalmitis

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Objectives: Nocardiosis is a localised or disseminated infection caused by soilborne aerobic actinomycetes. The genus *Nocardia* currently contains more than 50 species that have not been subjected to the same level of analysis by phenotypic and molecular methods. Apart from the two species most frequently isolated, *N. asteroides* and *N. brasiliensis*, new species recently characterised have been reported as human pathogens. We report the first case of scleral buckle associated *N. puris* endophthalmitis in an 80-year-old male.
Methods: The patient was initially treated in 1986 for retinal detachment by scleral buckle surgery in Vienna, Austria. Twenty years later, he was referred to the 2nd Department of Ophthalmology, Hellenic Red Cross Hospital, Athens, with a severe inflammatory reaction in the anterior chamber and vitreous body. After silicone sponge explant removal, the microbiological diagnosis of a *Nocardia* strain was made by conventional identification procedures (Gram stain, modified Kinyoun stain, colonial morphology, nitrate reductase and urease production, esculin hydrolysis) and 16S rRNA gene sequencing and phylogenetic analysis, according to the protocol described by Hiraishi et al. (1992). The primers used for amplification were the forward primer F16S (5′-AGAGTTTGATCCTGGCTCAG-3′, position 8–27 on the 16S rRNA gene of *E. coli*) and the reverse primer R16S (5′-GGTTACCTTGTTACGACTT-3′, position 1510–1492).
Results: Pairwise comparison of the obtained 16S rRNA gene sequence with the respective sequences of all bacterial species and strains available in GenBank database revealed that the *Nocardia* isolate of the present study had a 99% sequence similarity with *Nocardia puris*. The infection resolved after systemic administration of moxifloxacin hydrochloride (400 mg po q24 h for >1mo), and topical-eye combination therapy with antibiotics and corticosteroids.
Conclusion: To our knowledge, this is the first report of *N. puris* endophthalmitis, worldwide. Postoperative endophthalmitis may occur weeks to years following surgery, but such a delayed infection in an immunocompetent patient was likely due to the low-virulence of the organism introduced at the time of surgery. Surgical therapy remains the cornerstone of therapy, however, antimicrobials, particularly newer fourth generation fluoroqunilones, seem to play an important role in clinical recovery.

P1851 Clinical features of spondylodiscitis: a four-year survey

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Objectives: The spinal column diseases take a prominent place in the morbidity of adults. Spondylodiscitis represents an inflammatory process, localised in the bodies of spinal vertebrae and intervertebral disk. This research aimed to determine the most common complaints and clinical features of patients with spondylodiscitis as well as to analyse the correlation between the disease outcome and the beginning of therapy.
Methods: retrospective analysis of medical documentation was used, which encompassed 40 patients treated for spondylodiscitis at the Clinic for infectious diseases, Clinical Centre of Vojvodina, during the last four years. The diagnosis was confirmed in all patients using magnetic resonance (MR) imaging of spinal column.
Results: The average age of the patients was 58.5 years. In most of them, the inflammatory process was located in lumbar region (67.5%), then in lumbar sacral segment of spinal column (17.5%), while in the minority of the patients it was located in thoracic (7.5%) and cervical segment. The time before onset of the disease and establishing the diagnosis and beginning the therapy varied from one month (45%) to six and more months (17.5%). In all the patients the laboratory findings showed signs of inflammatory process. The aetiological agent has not been proved in 80% of them, in 12.5% it was tuberculosis of spinal column and in 7.5% brucellosis was found out. All patients were subjected to conservative-parenteral antibiotic therapy (tuberculostatic in case of tuberculosis). A combination of two or three antibiotics during six weeks to six months (51.6 days in average) was applied. Most often used antibiotics were the third generation cephalosporins, vancomycin, metronidazole, fluorochinolones, and aminoglycosides. In 4 (10%) patients surgical treatment was necessary in addition to the conservative one. The results

of the therapy were followed out by clinical examination, as well as by laboratory findings and MR imaging.
Conclusion: The outcome of the disease was favourable in most of the patients – recovery or recuperation with lesser or major sequels. The sooner the diagnosis was established, the more favourable outcome was. It is important to consider this disease because timely diagnostics and therapy shorten the duration of the illness, reduce the costs of treatment and minimize the possibility of sequels.

P1852 Laboratory acquired brucellosis – A review of published reports 1950–2007

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Objectives: To review the follow up and management of people exposed to brucellosis in laboratory settings.
Methods: Comprehensive review of all individual and aggregated case reports of laboratory exposures to brucellosis since 1950, identified through Medline, Scopus, reviews and textbooks. Mesh search terms combined "brucellosis", "laboratory infection", "occupational exposure", "risk factors", "drug therapy".
Results: 8 reports described the outcomes after multiple staff exposures in single laboratory incidents, with attack rates in personnel ranging from 0–31%. Approaches to post exposure risk assessment and prophylaxis (PEP) vary greatly, but no cases occurred after receiving any type of PEP. Compliance with prolonged post event surveillance is poor, and dual therapies for PEP are not well tolerated. 59 cases of laboratory acquired brucellosis were identified, of which 39 were due to *Brucella melitensis*: all except one had clinical symptoms at the time of diagnosis. Risk factors included aerosolisation and inadequate safety practices, especially before the organism had been identified. Many treatment regimens and durations were used, reflecting changes in international guidelines over time. 17 (29%) received monotherapy. The most common regimen was rifampicin and doxycycline for 6 weeks. 11 (19%) cases had relapse, 3 to 38 weeks after therapy finished. Cotrimoxazole was usually used in pregnancy. Of 6 pregnant women, only 3 delivered at term.
Conclusions: Brucellosis remains a hazard for laboratory staff. There is no clear evidence base for the correct PEP regimen but monotherapy is better tolerated. A standardised approach to risk assessment, surveillance and PEP is being developed, based on this literature review and an ongoing survey of recent laboratory experiences in the UK.

P1853 Pandrug-resistant *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* infections: characteristics and outcomes in a series of 28 patients

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Objectives: Clinical data regarding nosocomial infections due to pandrug-resistant (PDR) Gram-negative bacteria and their outcome are scarce. We describe our experience on the outcome of PDR *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* infections.
Methods: Retrospective, observational study of hospitalised patients at a tertiary-care hospital during a 17-month period (1/1/2006–31/05/2007).
Results: Twenty-eight patients with isolation of PDR Gram-negative bacteria were identified; 24 patients had infection and 4 colonisation. The most frequent PDR isolate was *K. pneumoniae* (24 isolates); three isolates were *P. aeruginosa* and 2 *A. baumannii*. The site of infection was central venous catheter related in 6 out of 24 patients, bacteraemia 5, respiratory system 5, surgical site 4, urinary system 2, ascitic fluid 1, and central nervous system 1. Twenty of 24 patients with infection received an antibiotic regimen containing colistin (in combination with meropenem in 8). The overall in-hospital mortality was 41.6% (10/24); 8 patients died because of the PDR infection (infection-related mortality 33.3%). Significant comorbidity was present in patients that died but also in survivors.
Conclusions: PDR Gram-negative bacterial infections are associated with considerable mortality, though not that high one would think of, given the fact that the isolates were resistant to all tested antibiotics, including polymyxins. Antibiotics that are ineffective in vitro may prove life saving for some of these patients, especially combination regimens containing colistin. However, comparative studies are necessary to estimate the attributable mortality of these infections and the appropriate treatment

P1854 Isolation of carbapenemase-producing *Pseudomonas otitidis* from a patient with chronic otitis media

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Objectives: To report otitis media due to metallo-β-lactamase (MBL)-producing isolate of *Pseudomonas otitidis*.
Methods: A 53-year-old man visited a Korean Eye and ENT hospital in April 2005. The patient had chronic otitis media since childhood. At the time of the visit, otalgia was absent but purulent discharge from perforated tympanic membrane was present. Prulent material was cultured and susceptibility was determined by tests of CLSI disk diffusion, broth microdilution and E-test. 16S rRNA gene sequencing was used to identify the species.
Results: A culture of the purulent material yielded two isolates, identified as *Pseudomonas aeruginosa* (PAE) by conventional tests, of which one was resistant to imipenem (IPM) and meropenem (MICs, >32 ug/ml) but susceptible to aztreonam, ceftazidime, cefepime, cefpiramide, cefoperazone-sulbactam, piperacillin, pipracillin-tazobactam, ciprofloxacin and trimethoprim-sulphamethoxazole (SXT). The IPM-resistant isolate was positive for MBL activity by the IPM-disk Hodge, and an IPM-EDTA plus SMA double disk synergy test. IPM-hydrolysing activity was detectable in a crude cell extract, independently of carbapenem exposure. However, PCR was negative for IMP-1, VIM-2 and SIM-1 genes, which were the only MBL genes reported in Korea at that time. Tests performed to investigate possible misidentification showed that the isolate was an oxidase-positive glucose nonfermenter, but pyocyanin and fluorescein production were negative. Many of the biochemical tests were inconsistent for PAE or *Stenotrophomonas maltophilia*. ID32 GN failed to identify the isolate. 16S rRNA gene sequencing identified it as *P. otitidis*, a new species causing otic infections, first isolated in the U.S.A. and reported in 2006. The patient was successfully treated with SXT and topical ofloxacin.
Conclusion: To our best knowledge, this is the first report of a *P. otitidis* infection outside the U.S.A. This observation underscore the possibility that some PAE isolates from otic infections could actually be *P. otitidis*. The mechanism of carbapenem resistance of this isolate, possibly due to production of a narrow-spectrum MBL, is under investigation. The clinical significance of the peculiar susceptibility pattern needs further study.

P1855 Microbiological documentation of *Pseudomonas aeruginosa* in bone and joint infections: analysis of 79 cases from a reference centre on a myoskeletal infections in Greece

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Objectives: *Pseudomonas aeruginosa* is not a frequent pathogen in bone and joint (BJ) infections. In the current study, clinical characteristics and outcome of patients treated for *P. aeruginosa* BJ in our outpatient clinic from 1999–2007 were analysed.
Patients and Methods: All cases of BJ infections that were clinically, radiologically, laboratory and microbiologically assessed, were retrospectively analysed via an electronic data-base registry. From a total of 574 records, 79 (13.7%) were microbiologically documented (cultures from pus, intra-operative tissues, bone and foreign material samples) as BJ infections caused by *P. aeruginosa*. Treatment success

was the clinical, radiological and microbiological resolution of the BJ infection while failure referred to all cases that did not fit this definition. Demographics, surgical operations, antimicrobial treatment outcome and infection relapses were evaluated.
Results: Male (n = 51, 64.5%) mean age 47.5 (SD+17.2) and the history of a car accident related to the BJ infection (n = 38, 48%) characterised *P. aeruginosa* BJ infections. These parameters (including age >65 years) did not correlate with poor outcome (p = 0.4, p = 0.5, p = 0.6 respectively). However, the presence of an infected orthopaedic device (n = 20/79) either arthroplasty or osteosynthesis, might slightly influence outcome (success 14/20 with and 27/59 without an infected device, p = 0.1). Surgical debridement predicted a successful outcome (21/36 vs 20/42, p = 0.04). The administration of high doses of ciprofloxacin (1500 mg or 2000 mg/24 h) that suggested a *P. aeruginosa* isolate susceptible to quinolones and offer the chance of per os treatment, is a strong predictor of good outcome (36/66 vs 5/11, p = 0.002). Neither dosing nor duration of antimicrobial treatment (mean 11, SD+ 30.3 months) seemed to influence outcome (p = 0.4 and p = 0.7 respectively). In a long-term follow-up (median 29 months, range 2–60) only 3 relapses of the infection were recruited. Overall success rates reached 50% (39/79).
Conclusions: 1. P aerugionosa BJ infections are often post-traumatic 2.Surgical debridement is the cornerstone of treatment in these infections along with appropriate antimicrobial therapy. 3 *P. aeruginosa* BJ infection is characterised by a high incidence (50%) of treatment failure despite adequate surgical and conservative therapy.

Brucellosis

P1856 Clinical manifestations and outcomes of therapy in 44 cases of brucellar spondylitis

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Objectives: For assessing the clinical features and outcome of treatment in brucellar spondylitis
Methods: This study was conducted on 44 cases with brucellar spondylitis from April 1998 through September 2007.The clinical manifestations and outcome of treatment were recorded
Results: Twenty six (59.1%) cases were male. the mean age of the patients was 47.9±16 years. Back pain, sweating, fever and artheralgia were the most clinical findings. The disease was acute in 23 (52.3%), sub-acute in 18 (40.9%) and chronic in 3 (6.8%) of the cases. Brucellar spondylitis was seen in 38 (86.4%) cases in lumbar and lumbo-sacral in 3 (6.8%) cases in cervical regions. Twenty cases were treated with doxycycline plus rifampin for 4 months. Relapse was seen in 1 (5%) case. Relapse was seen in 1 (9.1%) of 11 cases who were treated with cotrimoxazole plus rifampin for 4 months. Relapse was not seen in 15 cases treated with streptomycin for 14 days plus doxycycline and rifampin for 4 months
Conclusion: Brucellar spondylitis may develop in any sites of the spine mostly in the lumbar region. Streptomycin for 14 days plus doxycycline plus rifampin for four months may be better regimen for therapy of brucellar spondylitis.

P1857 Brucellosis: an evaluation of 100 cases in Central Anatolia

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Objectives: Brucellosis is a frequently encountered zoonosis in various regions of the world, including the Mediterranian region. It is endemic in some regions of our country, especially in the central Anatolian region. In our study we investigated restrospectively the brucellosis cases, in our hospital, our aim was to share the clinic, laboratory and therapy findings and to investigate their relationship with relapse rates.
Methods: A total of 100 patients diagnosed as brucellosis in our clinic between 2001 and 2006 were reviewed. The diagnosis was made by clinical symptoms together with laboratory results; titer >1:160 in the standard tube agglutination test (STA) and/or isolation of brucella species from blood and other bodily fluids or tissues. The Chi-squared test, Student's, Mann-Whitney U test, Kolmogorov-Smirnov Test were used for statistically analysis.
Results: In the 6 year study time 100 cases of brucellosis were established. 66 were male, and 34 were female. Among the 100 cases diagnosed as brucellosis, a total of 70 complications were found in 54 patients. The most common complications were found in osteoarticular, gastrointestinal and genitourinary systems as showed in the table 1. During follow up after treatment, relaps were seen in 28 cases. When the patients' demographic, clinical, and laboratory findings were evaluated, in relation to relapse, family history of brucellosis, living in rural areas and the presence of complications were related to higher relapse rates. Comparison of 100 patients with brucellosis with or without relapse are given in table 2. The most frequently used antibiotic combinations were; 77 doxycycline+rifampin, 10 doxycycline+streptomycin, 10 rifampin+ciprofloxacin, 1 doxycycline+rifampin+ciprofloxacin, 1 doxy-cycline+gentamycin, 1 doxycycline+streptomycin+ciprofloxacin. There was no significant statistical difference in the relapse rates according to the antibiotic used (p = 0.697). There was no mortalities.
Conclusion: In our study, we found that relapse was related to positive family history, living in rural areas, and the presence of complications. Brucellosis, still preserves its importance in our country, as it is endemic, shows a great variety of symptoms, can affect different systems, and there is still therapy handicaps and relapses are seen frequently. The efforts shown for the diagnosis and therapy should also be shown for protection and control strategies.

Table 1. Comparison of 100 patients with brucellosis with or without relapse

	Relapse (n = 28)	Without relapse (n = 72)	*p*
Age, years (mean±SD)	50.9±19.1	45.3±16.9	0.155
Male gender	16 (53.8)	50 (36.6)	0.352
Positive family history	11 (30.8)	13 (12.6)	0.049*
Animal contact	15 (56.4)	33 (40.9)	0.637
Rural area	19 (66.7)	30 (46.4)	0.033*
Clinical type			
acute	20 (61.5)	55 (79.8)	0.931
subacute	5 (10.3)	10 (12)	
chronic	3 (28.2)	7 (8.2)	
Hepatomegaly	13 (35.9)	23 (20.8)	0.261
Splenomegaly	5 (15.4)	5 (7.1)	0.137
Lymphadenopathy	1 (5.1)	1 (2.9)	0.484
Complications	21 (69.2)	33 (44.8)	0.016*
Sedimentation rate (>20 mm/h)	15 (38.5)	25 (32.2)	0.134
CRP	13	29	0.738
Anaemia	9 (28.2)	15 (14.7)	0.353
Leukocytosis	4 (15.4)	8 (8.7)	0.735
Leukopenia	3 (12.8)	8 (7.1)	1.000
Thrombocytopenia	5 (12.8)	8 (12.6)	0.508
Elevated AST (>40 IU/L)	15 (35.9)	23 (23.5)	0.077
Elevated ALT (>40 IU/L)	14 (33.3)	25 (22.9)	0.239
Standard tube agglutination (median, range)	320 (160–1280)	240 (160–1280)	0.536

*Significant.

P1858 **Focal organ involvement in a series of 99 brucellosis patients**

T. Turunc, Y. Demiroglu, H. Aliskan, S. Colakoglu, H. Arslan (Ankara, TR)

Objectives: Brucellosis is a zoonosis which affects all organs and systems and causes severe complications. The aim of this study was to evaluate demographic features, clinical findings and treatment outcomes of brucellosis with focal involvement.

Methods: This is a prospective study and included 99 patients with brucellosis aged over 15 years and presenting to our clinic between January 2003 and July 2007.

Results: The study included 99 patients, of whom 50 (50.5%) were female and 49 (49.5%) were male. They were aged between 15 and 77 years with a mean of 50.1 years. Out of 99 patients, 69 (69.6%) had acute brucellosis, 24 (24.2%) subacute brucellosis and 6 (6.2%) chronic brucellosis. Eighty-two patients (82.8%) had a history of consumption of unpasteurised milk and dairy products and 29 patients (29.2%) had a history of stockbreeding. In addition, 16 patients (16.1%) had a history of brucellosis at least one year before. The most frequent complication was musculoskeletal involvement (n = 80), followed by endocarditis (n = 9) and peritonitis (n = 1). The most frequent musculoskeletal involvement was spondylodiscitis (n = 50;50.5%), followed by sacroileitis (n = 21;21.2%) and peripheric arthritis (n = 9;9.09%). *Brucella melitensis* was isolated in blood cultures of 42 patients, spinal fluid cultures of one patient, ascites fluid culture of one patient, joint fluid culture of one patient and psoas abscess culture of one patient.

Conclusion: Brucellosis is a disease presenting with various clinical pictures and severe complications and difficult to diagnose. Therefore, caution should be exerted with focal organ involvement and brucellosis cases should be followed at regular intervals in regions where the disease is endemic. In addition, it should be kept in mind that *Brucella* spp. can be isolated in all sterile bodily fluids and that specimens should be obtained from appropriate sites.

P1859 **Four cases of sickle cell anaemia accompanied by brucellosis causing pain crisis**

T. Turunc, Y. Demiroglu, E. Kizilkilic, H. Aliskan, S. Colakoglu, H. Ozdogu, H. Arslan (Ankara, TR)

Objectives: Pain crisis which frequently appears in cases of sickle cell anaemia causes such complaints as severe pain in the extremities and the back and fever at the beginning. Among the factors which trigger pain crisis are extreme changes in temperature and stress. However, the cause is usually unknown. The aim of this study was to determine clinical features, laboratory results and treatment outcomes of sickle cell anaemia accompanied by brucellosis causing pain crisis. To our knowledge, there have not been such cases in the literature.

Methods: Four patients with sickle cell anaemia diagnosed with acute brucellosis when under supervision for pain crisis in Haematology Department between June 2004 and September 2006 were consecutively enrolled in the study.

Results: Out of four patients, two were male and two were female. The patients were aged 26–46 years. All patients had a history of consumption of unpasteurised milk and milk products. The most frequent complaints were fever (4/4), severe joint pain (3/4) and severe muscle pain (3/4). Brucellosis agglutination test was positive at 1/1280 in all patients and blood cultures showed *Brucella melitensis* in two patients. Only one patient had focal organ involvement in the form of epidural abscess in the lumbar region. This patient was administered doxycycline 200 mg/day (p.o.), rifampicin 600 mg/day (p.o.) and cotrimoxazole 800 mg/day (p.o.) for 6 months. The others patients were treated doxycycline 200 mg/day (p.o.), rifampicin 600 mg/day (p.o.) for 45 days. All patients recovered following completion of the medical treatment. The patient with epidural abscess had a relapse about 8 months after completion of the treatment. The other patients had no relapses.

Conclusion: High fever and severe joint pain are the most frequent signs of pain crisis in sickle cell anaemia. However, those symptoms may indicate acute brucellosis as in our series. Therefore, brucellosis should be kept in mind in patients with sickle cell anaemia presenting with high fever in regions where brucellosis is endemic and appropriate tests and blood cultures should be performed for the diagnosis of brucellosis.

P1860 **Brucellosis: a cause of peripheral neuropathy**

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Objectives: Brucellosis is a common infectious disease in Mediterranean countries. Central nervous system (CNS) involvement is rare, occurring <5% of adult cases. We evaluated the peripheral nerve involvement in patients with brucellosis.

Methods: Thirty-one patients diagnosed with brucellosis were included in this study. All patients were investigated for paraproteinaemia, endocrinopathy, vitamin B12 and folate deficiencies, connective tissue disorder, infections other than brucellosis, alcohol abuse, hepatic and renal failure. Four patients were excluded from the study because of B12 deficiency and a history of diabetes mellitus. The remaining 27 patients were included. Average age was 45.19 ± 16.21. Seventeen of these patients were male and the other 10 were female. All patients underwent nerve conduction and electromyography (EMG) studies and 20 normal healthy subjects were used as a control group.

Results: Among 27 patients of brucellosis, six of them had CNS involvement. In electrophysiological examinations, sensory-motor neuropathy was determined in nine patients who had abnormal neurological examination. One patient had also polyradiculoneuritis together with peripheral neuropathy. The other eight patients had hypoactive deep tendon reflexes and paresthesia in distal extremities. All patients had received combination treatment (rifampin and doxycycline and/or ceftriaxone) for 3–9 months. After six months of the treatment, nerve conduction studies and neurological examination were nearly normal in all patients. The EMG findings of 18 patients, who had no prominent peripheral neuropathy and normal neurological examination, were compared with normal healthy subjects. The motor conduction velocities of median ($p < 0.05$), ulnar ($p < 0.05$), peroneal ($p < 0.001$) nerve was decreased. F wave latencies was prolonged in posterior tibial and peroneal nerve, distal latency was also prolonged in posterior tibial nerve in brucellosis patients compared to normal ($p < 0.05$). Sensory conduction velocities of median ($p < 0.001$), ulnar ($p < 0.05$) and sural ($p < 0.05$) nerve was also decreased. There were no statistically differences in terms of age, sex and duration of the symptoms between the patients with and without peripheral neuropathy.

Conclusions: Clinical or subclinical sensory-motor peripheral neuropathy may be observed in patients with brucellosis. Consequently brucellosis may be considered as a cause of peripheral neuropathy especially in endemic areas.

P1861 **Copper and zinc plasma level in brucellosis patients in Iran**

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Objectives: Brucellosis which is a zoonotic disease is a common endemic disease and health problem in Iran. Based on multiple studies, copper and zinc have influence on immune system. In other studies it has been shown that plasma levels of these trace elements in patient with Brucellosis are changing. The aim of this study was to measure the serum zinc and copper level in patients with brucellosis and in comparison with healthy individuals.

Methods: In this cross sectional study the plasma level of zinc and copper of brucellosis patients were measured and compared to the level of healthy individuals.

Results: Forty three patients (34 men and 9 women) with brucellosis matched with 43 healthy (34 men and 9 women) individuals were included in two teaching hospital in two cities of Iran in 2006. The mean age of patients were 40.14 years old (range 14–60 years old) and the mean age of healthy individuals were 40.3 ± 15.89 years old (range 14–60 years old). The plasma level of copper was found to be

significantly higher in patients with brucellosis than healthy individuals (160.84±54.61 vs 101.74±27.37 μg/dl). The plasma level of zinc was less than healthy individuals (91.47± 46.91 vs 107.19±35.18 μg/dl) but was not statistically significant. Zinc plasma concentration was lower in female brucellosis patients compared with healthy female individuals (80.33± 44.2 vs 119±28.17 μg/dl).

Conclusion: Plasma zinc and copper level showed significant alterations in patients with brucellosis compared with healthy persons and there is influenced of gender on it. It is still not clear that these changes are result of the disease or these changes predispose individuals to infection.

P1862 ***Brucella melitensis*** **DNA persistence in patients with chronic brucellosis**

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Objective: The aim of this study was to evaluate the evolution of bacterial load (*B. melitensis* DNA copies/ml sample) at baseline, during therapy and post-therapy follow-up in patients with chronic brucellosis.

Methods: For that purpose, we applied the Real-Time Quantitative Polymerase Chain reaction technique (QPCR) described by our team in a previous study [1]. We analysed 125 whole-blood and 122 serum samples from 10 patients using QPCR. Blood culture and serological tests (rose bengal stain, serum agglutination test (SAT) and Coombs test) were performed for each sample.

Results: The mean bacterial load (± SD) at baseline was 634±1403 copies/ml and 1738±4813 copies/ml for whole-blood and serum samples, respectively. Eight patients received therapy, doxycycline alone or in combination with rifampin or cotrimoxazol. The duration of therapy ranged from 90 to 430 days (mean 140 days). Therapy improved symptoms in all patients. During the first month of the treatment period the bacterial load declined to 19±39 copies/ml and 26±102 copies/ml for whole-blood and serum samples, respectively. Thereafter, there was an increase in load until the end of the therapy (1507±3787 copies/ml blood and 2669±7790 copies/ml serum).

In the first 3 months of the post-therapy follow-up, the bacterial load decreased again to 29±126 copies/ml and 222±882 copies/ml for whole-blood and serum samples, respectively. Only six patients were followed for more than 12 months. At the end of the follow-up period, the bacterial load amounted to 2917 ±11354 and 2549±8691 copies/ml in whole-blood and serum samples respectively for 33% (2/6) patients.

In 4 patients, although presenting clinical features of brucellosis, the samples collected were negative for all the classical serological procedures. However *B. melitensis* DNA was still detectable by QPCR.

Conclusion: In conclusion, we detect fluctuating *B. melitensis* DNA levels in patients with chronic brucellosis during the course of the disease despite positive clinical response to therapy. We found a subgroup of brucellosis patients showing negative serology but presenting detectable levels of *B. melitensis* DNA assed by QPCR.

Reference(s)

[1] Navarro et al, Clin Infect Dis 2006 May 1; 42(9):1266–73.

P1863 **Quantification of *Brucella melitensis* DNA in patients with brucellosis**

E. Navarro, M.J. Castaño, E. Alfaro-Martínez, J.C. Segura, S. Lorente, L. Garijo, C. Romero, J.L. Beato, J. Solera (Albacete, ES)

In the present study we describe the evolution of bacterial load (*B. melitensis* DNA copies/ml of blood) at baseline, during therapy and post-therapy follow-up in patients with brucellosis. Patients were assigned to two different groups according to the evolution of the disease after treatment: non-relapse patients (group I) and relapse patients (group II).

To this aim, we applied the Real-Time Quantitative Polymerase Chain reaction technique (PCRQ) described by our team in a previous study [1]. Peripheral blood samples from 14 non-relapse (N = 103) and 8 relapse (N = 90) patients were analysed using PCRQ. Blood culture and serological tests were performed for each sample (rose bengal stain, serum agglutination test (SAT) and Coombs test).

The evolution of the bacterial load was similar in both groups during the acute episode. Basal load was 1619±2076 copies/ml (mean, s.d.) and 2778±3909 copies/ml for groups I and II, respectively. Bacterial load decreased during the first month of treatment in both groups, but the difference was statistically significant only for the non-relapse group ($p = 0.03$). Between day 29 and the end of treatment, bacterial load increased in mean value (930±2105 copies/ml and 677±1115 copies/ml for groups I and II, respectively) without reaching statistical significance ($p = 0.205$).

Follow-up of group I patients showed a decrease in bacterial load down to 120±274 copies/ml during the first three months. Thereafter, there was a mild increase in load until the ninth month of follow-up (220±475 copies/ml). At the end of the follow-up period, the bacterial load amounted to 22±34 copies/ml for 50% of non-relapse patients.

In relapse patients, bacterial load initially decreased to 83±78 copies/ml during the first two months of follow-up before relapse occurred. At the time of relapse, the bacterial load increased to 4626±7013 copies/ml, decreasing progressively with the re-instated treatment until the end of the follow-up period.

In patients with brucellosis, we were able to intermittently detect *Brucella melitensis* DNA after the treatment period, despite positive clinical response to therapy. We have been unable to determine the level of basal load that may be predictive of relapse. However, we found that only the non-relapse patients showed a significant decrease in the bacterial load during the first month of treatment.

Reference(s)

[1] Navarro et al, Clin Infect Dis 2006 May 1; 42(9):1266–73.

P1864 ***Brucella*** **endocarditis in Iran, a treatable disease**

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Objectives: Brucellosis is a worldwide zoonosis caused by pathogens of the genus *Brucella*. Endocarditis is a rare focal complication of this disease with destructive valvular lesions and the most common cause of brucellosis-related mortality. In this study we have presented 9 cases of brucella endocarditis who were admitted in infectious diseases wards of Imam Khomeini Teaching Hospital in Iran from 2000–2005.

Methods: The charts of all cases with diagnosis of brusella endocarditis were reviewed to assess their clinical course, treatment and outcome.

Results: There were 9 patients with diagnosis of brucella endocarditis in period of 2000–2005.The mean age of the patients were 38.11 years (range14–69 years), among them, 7 were men and 2 were women. Seven patients (77.7%) had consumed nonpasteurised dairy products and 1 had occupational contact with goat. Underlying cardiopathy was present in 3 patients (33.3%). The median duration of the symptoms prior to diagnosis was 5.33 months. All of the patients had fever. Endocarditis involved the aortic valve in 6 cases (66.6%), the mitral valve in 2 cases (22.2%), and the aortic plus mitral valve in 1 case (11.1%). Serologic tests were positive in eight (88.8%) and blood cultures were positive in 2 patients (22.2%). Aortic valve replacement surgery was undertaken in 5 patients (55.5%). Radial artery aneurismectomy was done in one patient. One patient died because of arrhythmia. All other 8 patients improved and were symptom free at the time of discharge from hospital. All patients received triple therapy with different appropriate antibiotics.

Conclusion: Brusella endocarditis which is the main cause of mortality in brucellosis could be a treatable disease if diagnosed and managed in a proper time. Diagnosis is usually based on epidemiologic, clinical and para clinical parameters. A combination of medical and surgical intervention is usually the treatment of choice in these patients.

P1865 **Neurobrucellosis: the evalution of a rare presentation of brucellosis from a tertiary care centre at Central Anatolia**

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Objectives: Brucellosis is a zoonotic and multisystem disease which is highly endemic in Turkey similiar to other Mediterranean countries. Any organ or system of the body including nervous system can be involved. The aim of this study is to describe our experience in the diagnosis, treatment, and final outcome of the patients with neurobrucellosis who were diagnosed and treated in our clinic.

Patients and Methods: This study was conducted at Erciyes University Gevher Nesibe Hospital, a 1300-bed tertiary referral centre in central Anatolia. We retrospectively reviewed the records of the Department of Infectious Diseases to identifiy the patients aged $\geqslant$16 years with neurobrucellosis from January 1997 to December 2006. Neurobrucellosis was diagnosed by the following criteria: symptoms or clinical findings concordant with neurobrucellosis, isolation of *Brucella* from the cerebrospinal fluid (CSF) or/and demonstration of antibodies to *Brucella* in CSF (at any titer), the presence of any abnormality in CSF (pleocytosis, increased protein levels and decreased glucose levels) with positive serology for brucellosis.

Results: Nine hundred and seventeen patients with brucellosis were admitted to our department during 10-years. Thirty-six patients were diagnosed as neurobrucellosis. (Table 1). Headache and fever were the most common symptoms. Neck stiffness was present in 25 of the patients and the 69.4% of the patients were diagnosed as acute meningitis. Nervus abducens involvement was detected in two patients. Motor deficit was determined at the lower extremities at five and upper extremities in two patients. Hemiparesis was assigned in two patients. Urinary and gaita incontinence was observed in two patients.

Brucella spp. was isolated from the blood of nine of patients and from the CSF of 11. The median serum standart tube agglutination (STA) value of the patients was 1:320 and positive in 30 patients. The median CSF STA titer was 1:20 and positive in 21 patients.

Table 1. Features of the patients with neurobrucellosis

	Number of patients
Female/Male	17/19 (47.2/52.8%)
Age (mean, years)	36.5±16.4 (range, 18–77)
Duration of symptoms (mean, days)	64.7±111.3 (range, 2–545)
Concomitant disease	7 (19.4%)
Diabetes mellitus (DM)	1
DM + chronic renal insufficiency	1
Congestive heart failure	2
Acute myeloid leukaemia	1
Chronic bronchitis	2
Clinical spectrums	
Acute	26 (72.2%)
Subacute	8 (22.2%)
Chronic	2 (5.5%)
Meningitis	25 (69.4%)
Meningoencephalitis	11 (30.6%)

Doxycycline (po) plus rifampin (po) with ceftriaxone (iv) was the most common choice for the treatment. The duration of treatment was three months in 33 patients and four months in three patients. Overall mortality was 8% in this study and none of the patients died due to neurobrucellosis. Relaps occurred in one patient.

Conclusion: Neurobrucellosis presents with hetoregenous clinical signs and attrending physicians should remind the colourfull picture of neurobrucellosis in endemic areas.

P1866 **Determining in vitro synergistic activities of tigecycline with several antibiotics against *Brucella melitensis* using chequerboard and time-kill assays**

H. Aliskan, F. Can, M. Demirbilek, S. Colakoglu, H. Arslan (Ankara, TR)

Objectives: Antimicrobial therapy of *Brucella* spp. infection is difficult because there are relatively few effective treatment regimens, and single-agent therapy frequently fails clinically. Assessing the killing activity and the effect of combination therapy (synergistic or antagonistic) is important for antibiotics used to treat brucellosis.

Methods: In this study, the in vitro activity of tigecycline in combination with gentamicin, streptomycin, rifampin, co-trimoxazole, levofloxacin, and minocycline was investigated with the chequerboard method in 16 *Brucella melitensis* isolates. The time-kill method was used to determine bactericidal activities of combinations of tigecycline with rifampin, gentamicin, and levofloxacin, which were found to have a synergistic effect in combination with tigecycline.

Results: Using the chequerboard method, combinations of rifampin, gentamicin, and levofloxacin with tigecycline yielded synergy in 31.2%, 18.9%, 50% of the isolates respectively. No synergism was observed between combinations of minocycline, streptomycin, and co-trimoxazole with tigecycline by the chequerboard method.

Time-kill results showed that tigecycline+gentamicin achieved the fastest complete killing within 6 h and created a synergy as shown by the chequerboard method. Although rifampin alone caused complete killing within 48 h at MIC, rifampin+tigecycline combination exhibited faster killing only within 24 h. In this study, the time-kill method showed that tigecycline in combination with levofloxacin had an antagonism, while the chequerboard method showed that they had synergy and no interaction effects. Tigecycline alone caused complete killing within 24 h at MIC and was the fastest acting agent.

Conclusion: The chequerboard method revealed that three antibiotic combinations (tigecycline+gentamicin, tigecycline+rifampin, and tigecycline+levofloxacin) had a synergistic activity. Although tigecycline+gentamicin and tigecycline+rifampin synergistic activities were confirmed by the time-kill method, the opposite effect was determined with tigecycline+levofloxacin combination. Several investigators have found discord between the chequerboard and time-kill methods, agreement ranges between 44% and 88% and the time-kill method to be a reliable predictor of in vivo synergy. It appears that the combinations of tigecycline+rifampin and tigecycline+gentamicin may be used to treat brucellosis.

Leishmanias

P1867 **Cloning and expression of truncated form of tissue plasminogen activator in *Leishmania tarentolae* as a new eukaryotic system**

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Objective: Tissue plasminogen activator protein(t-PA) was found as superior therombolytic agent for treatment of cardiovascular diseases such as acute myocardial infarction and efforts is currently focused to improve the t-PA molecule and thereby its pharmacokinetic properties. Reteplase (K2S) is a derivative t-PA that has a longer half-life and greater resistance to inhibitor than the natural t-PA molecule. The aim of this research is cloning and expression of K2S form of the t-PA cDNA in eukaryotic system *Leishmania tarentolae* which is recently has been introduced as a suitable host for expression eukaryotic genes.

Methods: cDNA of t-PA was made by RT-PCR from human blood cells. PCR with specific primers for producing truncated form of t-PA (K2S) were used. For introducing the truncated form of t-PA in *Leishmania tarentolae* cells, we constructed two plasmid pFXmlsap1.4sat-K2S and pFXmlsap1.4hyg-K2S, that each of them contains one antibiotic selection marker (neursothricin and hygromycin). After development of constructs electroporation was done on *L. tarentolae* cells for transfecting developed constructs.

Results: All constructs (pFXmlsap1.4sat-K2S and pFXmlsap1.4hyg-K2S) were confirmed by restriction analysis and PCR. After electroporation, screening of cells was done by selecting resistance clones to antibiotic in media. At this stage we confirmed that our cells carry truncated form of t-PA in the respected site SSurRNA (18s) and next step is to confirm the expression of gene.

P1868 Identification of *Leishmania* species isolated from human cutaneous leishmaniasis in Gonbad-e Qabus using a PCR method

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Objectives: To determine the epidemiological status of the cutaneous leishmaniasis outbreak, isolation and identification of the parasite using a PCR method in Gonbad-e Qabus County, north Iran, during 2006–2007.
Methods: Data was collected on the prevalence of scars and ulcers over a period of 16 months among 6990 inhabitants of five villages around Gonbad-e Qabus County. Cultured promastigotes were identified using PCR technique. ITS1 and ITS2 of Non Coding Transcribed region at ribosomal DNA of 46 *Leishmania* isolates were amplified and the PCR products were separated by electrophoresis in 2% agarose gel (200 mA, 140 V), visualised by staining with ethidium bromide, and photographed.
Results: Among 6990 inhabitants of 5 villages, 62.9% had scars and 1.5% had active lesions. Individuals 11 to 20 years of age were the most highly infected age group. All the parasite isolates were *Leishmania major*.
Conclusion: According to this study, cutaneous leishmaniasis due to *Leishmania major* is endemic in Gonbad-e Qabus County, north Iran.

P1869 Efficacy of amphotericin B and voriconazole against *Leishmania tropica* with microcapillary culture method

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Objectives: *Leishmania* parasites cause a wide range of human diseases from localised self-healing cutaneous lesions to fatal visceral disease. The causative agent of Cutaneous Leishmaniasis (CL) in southeastern Turkey is mainly *Leishmania tropica*. A sensitive microcapillary culture method (MCM) was developed for rapid diagnosis of CL by Allahverdiyev et al. The aim of this study is researching the effect of amphotericin B (AMB) and voriconazole (VOR) on *Leishmania tropica* (MHOM/ TR/EP39) using MCM in order to determine more effective alternatives to antileishmanial drugs which current being used for treatment.
Methods: In this study, the promastigotes these are growth in NNN medium and passaged RPMI-1640 medium (with L-glutamine) buffered with 25 mM HEPES and supplemented with 20% fetal calf serum, are inoculated in mixed solutions prepared with different drug concentrations and medium. Capillary tubes will load with medium and drug dilution. The ends of the capillary tubes were seal with heat-melted candle wax. All inoculated tubes incubated at +26ºC for 72 hours. End of time, the capillary tubes are cut off ends and promastigotes are counted on Thoma chamber. The 50% inhibitory concentrations (IC50) are calculated.
Results: The IC50 values for AMB and VOR were determined that 0.1475 μg/ml, 4.682 μg/ml, respectively.
Conclusion: In conclusion, it was found that AMB and VOR have got inhibitory effects of *L. tropica* promastigotes in vitro. IC50 values of VOR more than AMB, but due to their too few toxic effects than many antileishmanial drugs, they may be alternative treatments in future. In addition, the MCM has been used for the first time on drug investigations, which was seen to be valuable for in vitro drug tests of *Leishmania* as well as routine diagnosis of leishmaniasis. Further studies are needed to develop these results with in vivo CL animal models.

P1870 Induction of apoptosis by the triterpene saponin PX-6518 in promastigotes and amastigotes of *Leishmania infantum*

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Objectives: Current first-line medication of visceral leishmaniasis is increasingly faced with levels of resistance and new drugs are still needed to maintain adequate therapeutic options for the future (1). The 13,28 epoxy-oleanane saponin PX-6518 was shown to possess strong antileishmania properties in vivo (2). Because little is yet known about its mode of action, the induction of apoptosis was studied on promastigotes and extracellular and intracellular amastigotes of *L. infantum*. Miltefosin, for which apoptosis-inducing action was already demonstrated (3), was used as reference.
Methods: Early apoptosis, characterised by phosphatidylserine (PS) exposure from the inner to the outer side of the cell membrane, was evaluated by Annexin V-FITC/PI labeling (Ann/PI) in treated promastigotes and extracellular amastigotes. Differential sensitivities of axenically grown and fresh ex vivo amastigotes were compared. Microscopic and flow cytometric follow-up studies included DNA fragmentation in oligonucleosomal fragments using a Tunel assay. The effects on intracellular amastigotes were studied in J774 and primary peritoneal macrophages.
Results: Ann/PI evaluation of PX-6518 treated host cells demonstrated signs of apoptosis induction, whereas treatment of promastigotes and axenic amastigotes did not induce PS exposure. Evaluation of ex vivo amastigotes was hampered by the fact that a large proportion of untreated control cells were Ann-positive and PI-negative. This result confirms earlier reports of apoptotic mimicry (4). No additional effects of PX-6518 could be observed in the small fraction of Ann+, PI– amastigotes. These initial observations are now studied in greater detail using the Tunel assay.
Conclusion: Confirming earlier reports (5), features of early apoptosis were observed after treatment of macrophages with PX-6518. The effects on promastigotes and extracellular amastigotes were marginal. The results of ongoing follow up Tunel studies on the different *Leishmania* stages and host cells will be presented.

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P1871 Detection of *Leishmania* by PCR-oligochromatography with the "Leishmania OligoC-Test"

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Leishmaniasis is a vector born disease caused by protozoan of the *Leishmania* genus. At least 15 species can infect humans causing a broad spectrum of clinical manifestations like Visceral leishmaniasis (VL), cutaneous leishmanaisis (CL), muco-cutaneous leishmaniasis (MCL) and PKDL.
As no vaccine is available, the control of leishmaniasis relies on diagnosis and treatment of detected cases. Current diagnosis of VL is based on serology and is confirmed by microscopy (not very sensitive) or culture, which can take more than 4 days. Leishmanin Skin Test (LST), culture or microscopy of lesion biopsy are used for CL diagnosis. While quite sensitive, serology and LST cannot discriminate past and present infection. Moreover, in immunocompromised patients (eg. transplant, HIV/VL co-infection) the performance of serology is seriously affected.

PCR was shown to be a valuable tool for *Leishmania* diagnosis. Despite its high sensitivity, PCR is not routinely used in endemic countries mainly due to it's complexity, and the equipment costs that makes it unaffordable by laboratories with limited research facilities and resources. We have recently developed and evaluated the "Leishmania OligoC-Test", a rapid, easy-to-use and sensitive PCR-oligochromatography (PCR-OC) assay. Both PCR mix and detection device were designed to be used in limited facilities and resources settings.

The PCR mix contains an internal control (IC) allowing to highlight the presence of PCR inhibitors in the sample to validate any negative results, and dUTP to allow elimination of carry-over contaminations. The detection of PCR products (*Leishmania* amplicon and IC amplicon) is made by Oligochromatography, a one-step assay which combines hybridisation of specific probes coupled to colloidal gold and migration on a dipstick at 55ºC. The detection takes only 10 minutes and is made in closed tube. All the 12 *Leishmania* species tested with this assay are detected.

Up to 10fg (+/− 0.05 parasite) of genomic DNA are detected and it was possible to detected as few as 10 parasites in spiked blood. No cross-reactivity was observed with the other pathogenic *Trypanosoma* and bacteria we have tested. An evaluation on skin biopsies from Peru showed that most of the microscopy positive samples are detected with our assay. Leishmania OligoC-Test is a rapid, sensitive and specific assay. Its easiness and the fact that only a water bath is needed for post-PCR detection render it a valuable tool for a broad use.

P1872 Evaluation of anti-leishmanial efficacy by in vivo administration of herbal extract Artemisia auckery on *Leishmania major* in Balb/c mice

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Objectives: Cutaneous infection caused by protozoa the genus *Leishmania* are a major worldwide health problem, with high endemicity in developing countries including Middle East, Africa and Latin America. In the absence of a vaccine, there is an urgent need for effective drugs to replace and supplement those in current use.

Methods: We report in this study the in vivo efficacy of Artemisia auckery herbal extract on the *Leishmania major* cutaneouse infection in murine model in susceptible Balb/c mice. To carry out this investigation, mice were assigned to the 5 groups (each with 3 mice) as healthy negative control, saline-treated control, glucantim-treated and treated-groups with herbal extract. Experimental Leishmaniasis was initiated by the subcutaneous (s.c.) application of the 2×10^6 promastigotes of *L. major* (MRHO/IR/75/ER) into the basal tail of all groups except the healthy negative control group. The development of lesions was determined weekly by measuring the diameters. Inoculations of both Glucantime and herbal extract were carried out by subcutaneous injections once daily for 20 days. At the end of treatment course, all mice were killed humanely by terminal anaestheisa and target tissues including lymph node, spleen and liver from each mouse were removed and weighted and their impression smears were also prepared.

Results: The results indicated that our herbal extract was able to affect on lesion size, its performance and to prevent visceralisation of the parasite. This is the first report indicating visceralisation caused by the cutaneous form of *L. major* in the Balb/c mice. During this experiment no side effects were observed due to the application of herbal extract in the treated-mice. The impression smears showed a reduction of parasite burdens in spleen, liver and lymph node.

Conclusion: In comparison with glucantim; the present herbal combination was more effective on this murine Leishmaniasis, therefore it could be suggested as a substitute for glucantime in the treatment of Leishmaniasis for human and animal purposes.

P1873 Five cases of kala azar

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Objective: The aim of this study was to evaluate clinical features of kala azar, transmitted by sand flies. It is the second most frequent infectious disease caused by parasites following malaria.

Methods: Five patients with kala azar presenting to our clinic between April 2004 and May 2007 were consecutively enrolled in our study. The diagnosis of the disease was based on clinical findings and detection of amastigot forms of the parasite in bone marrow, liver and spleen specimens. Three patients were living in a rural area and two in an urban area.

Results: Out of five cases, three were female and two were male with a mean age of 47 years (range: 34–54 years). Two patients had a history of sand fly bites. The patients most frequently presented with fever, abdominal pain and weight loss. On history, one patient received treatment with steroids since he was found to have antinuclear antibodies during follow-up for accompanying hepatitis B and his exhaustion and weight loss increased. Meanwhile, the patient was found to have an increased erythrocyte sedimentation rate and globulin levels and underwent bone marrow biopsy. Obtained specimens showed *Leishmania* amastigots. The remaining two cases did not have any abnormalities in their bone marrow aspiration biopsies. However, they had widespread lymphadenopathy in the mediastinum and abdomen and fever of unknown origin. For these reasons, they underwent splenectomy. Obtained specimens showed *Leishmania* amastigots. All patients were administered liposomal amphotericin B 3 mg/kg daily for 5 days and once on days 14 and 21. None of the patients had relapses.

Conclusion: Kala azar may present with various clinical pictures in urban areas as well as in rural areas. We recommend that liver and spleen biopsies should also be performed since bone marrow biopsy may not show the parasite. Compared to conventional agents, liposomal amphotericin B seems to be a good treatment alternative in that it has fewer side-effects, requires a shorter treatment period and produces a satisfactory outcome.

P1874 Molecular epidemiology of cutaneous leishmaniasis in Jahrom, southern Iran

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Objectives: Cutaneous leishmaniasis is caused by a protozoan parasite called *Leishmania* spp. This infection is endemic in different parts of Iran including Jahrom County (Fars province). Since there were no data about the species of *Leishmania* in this county, we conducted this study to find the dominant species of the parasite.

Material and Methods: DNA extraction of positive giemsa-stained slides of 40 patients was used as a template for Nested-PCR.

Results: Findings of this study indicated the existence of both *L. major* & *L. tropica* in the area, although the dominant species was *L. major* (%85 of cases). The highest prevalence was in children under 10 years old. The most infected organs were hands. Most of the patients had one ulcer although up to 33 ulcers were observed.

Conclusion: This study showed the changes in the profile of the species of the parasite from *L. tropica* to *L. major* in Jahrom County.

Toxoplasmosis

P1875 Pyrimethamine/sulfadiazine and atovaquone impact maturation of antibodies and avidity against *Toxoplasma gondii* in a murine model of acute infection

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Objectives: Infection with *Toxoplasma gondii* during pregnancy may result in congenital transmission of the parasite from the mother to the

foetus. Infection is diagnosed using serological tests for IgG, IgM, and IgA antibodies. Avidity of IgG antibodies is regularly used to exclude acute infection. It has been suggested that antiparasitic treatment results in delayed maturation of IgG avidity. However, detailed studies on the effects of antiparasitic treatment on maturation of IgG avidity are lacking. We therefore investigated the maturation of antibodies and IgG avidity in a murine model of acute infection with *T. gondii*.
Methods: NMRI-mice were orally infected with 10 cysts (ME49) and treated with spiramycin, pyrimethamine plus sulfadiazine (pyr/sulf), azithromycin, or atovaquone (at dosages equivalent to patient dosages) from day 4 to day 10 p.i., or left untreated. Blood samples and brains were obtained at 14, 25, and 60 days (d) p.i. IgG, IgM, and IgA anti-*T. gondii* antibodies and IgG avidity were determined in serum by ELISA; numbers of cysts in brains were determined by immunohistochemistry.
Results: Concentrations of specific IgG, IgM, and IgA increased over time. IgG avidity also increased between d 14, 25, and 60 p.i. Treatment with pyr/sulf but not with spiramycin, azithromycin, or atovaquone significantly decreased concentrations of IgG but not IgM or IgA antibodies at 25 and 60 d p.i. ($p < 0.01$). This was paralleled by a significant decrease in cyst numbers in brains of mice treated with pyr/sulf but not other drugs. In contrast, atovaquone but not pyr/sulf or other drugs significantly decreased levels of IgG avidity at 60 d p.i. ($p = 0.02$).
Conclusion: Pyr/sulf but not other drugs currently used for the treatment of acute infection with *T. gondii* impacted the development of IgG antibodies against *T. gondii* and parasite numbers in a murine model of acute infection. Atovaquone but not pyr/sulf or other antiparasitic drugs impaired maturation of IgG antibodies. Future studies will have to investigate the impact of antiparasitic treatment on maturation of avidity in patients recently infected with *T. gondii*.

P1876 Use of a clinical laboratory database to estimate the incidence of *Toxoplasma gondii* infection among women of childbearing age: a longitudinal retrospective study

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Objective: To know the incidence of *Toxoplasma gondii* infection in women of reproductive age in Albacete, Spain.
Methods: We designed a longitudinal retrospective study of seronegative childbearing-aged women. This study was conducted in an area where prenatal serological screening for *T. gondii* infection is routinely performed. Serological testing within this area is centralised in a unique laboratory. We used the database of this central laboratory as the only source of information for this study. We included in the study all the women 14–44 years old who fulfilled all three criteria that follow: 1) they had at least two determinations of *T. gondii* IgG performed between Feb-2001 and Mar-2007, 2) the first determination of *T. gondii* IgG was negative, and 3) the last determination was separated from the first one by an interval of time of at least one year. The patients were classified in: 1) not infected, if all the determinations of *T. gondii* IgG were negative, 2) confirmed seroconversions, if the patient had a) at least two determinations of *T. gondii* IgG with negative result followed of at least two determinations with positive result or b) a determination of *T. gondii* IgG with negative result followed of at least two determinations with positive results for both *T. gondii* IgG and IgM, and 3) possible seroconversions, if the patient had at least one determination of *T. gondii* IgG with negative result followed of at least one determination with positive result but was not fulfilling the criteria of confirmed seroconversion. For every patient it was calculated a time at risk of acquiring the infection, which was the interval of time between the first one and the last determination of antibodies.
Results: We studied 2,416 women with a mean age of 29 years (SD = 5). The median time at risk was 35 months (interquartile range = 23–46), and the total sum of times at risk was 7,121 women-year. There were 5 confirmed and 3 possible seroconversions. None of these seroconversions was detected during a pregnancy. There were 0.7 confirmed and 0.4 possible seroconversions per 1,000 women-year. So, the true incidence was between 0.7 and 1.1 seroconversions per 1,000 women-year (that is, 0.5–0.8 per 1,000 women-9 months).
Conclusion: The incidence of *T. gondii* infection among women of childbearing age was low. Analysis of the laboratory database is a useful approach to monitor the incidence of infection in areas where prenatal screening for toxoplasmosis is performed.

P1877 Seasonal trends in acute toxoplasmosis in pregnancy in Upper Austria

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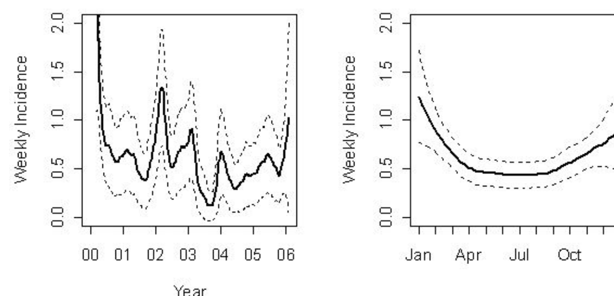
Objectives: Acute toxoplasmosis in pregnant women bears a risk of infection for the unborn that might result in moderate to severe ocular and neurological disorders. Secondary prevention using general screening during pregnancy and antimicrobial treatment is cumbersome, still disputed and carried out only in few European countries like Austria. Pregnant women should be advised to avoid risk factors for infection, especially eating of undercooked meat. The role of other important risk factors is less well understood. We seek to verify a recent observation from Slovenia suggesting a significant increase in the incidence of acute toxoplasmosis in pregnant women in winter.
Methods: We investigated retrospectively records for the largest statutory health insurance company in Upper Austria (OÖGKK) about toxoplasmosis screening of pregnant women participating in the mother-child-pass preventive programme. We used a general additive model with locally weighted regression to visualise the trend in weekly incidence of toxoplasmosis over time and for the estimation of the seasonal trend during a year. Since the overall number of investigations did not change over the studied period, we used Poisson distribution to model the incidence of toxoplasmosis cases. Acute toxoplasmosis has been regarded as certain in case of significant titre rise in indirect immunofluorescence test and positive IgM, and uncertain if diagnosis has been solely made by positive IgM and low IgG-avidity.
Results: From 01.01.2000 to 31.01.2005 there were 191 uncertain and 51 certain acute infections. There was a clear variability across time in uncertain infections, with a higher incidence during the winter months (Figure 1a). This was confirmed in the seasonal trend analysis, with a more than 2 times higher incidence during the winter months (Figure 1b). The results were similar when the analysis was restricted only to certain cases. In seroconversions during pregnancy (n = 45), the mean duration between the last seronegative and first seropositive examination was 102 days (range: 30–181 days).



Conclusion: We confirmed the trend observed in Slovenia using an independent sample from Upper Austria. Given the long times between examinations it can be expected that the observed peak in positive diagnoses in winter may actually reflect an increased incidence of infection in autumn. Further research should focus on risk factors that may explain the observed seasonal trends.

P1878 *Toxoplasma gondii* infection in centre of Portugal: seroprevalence during 2000–2006

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Toxoplasma gondii is an obligate intracellular protozoan that can infect all kind of birds and all mammals including humans and is common throughout the world. The prevalence varies according to social and cultural habits, pet cats in homes and geographic factors. Domestic cats are considered to be an important source of *T. gondii* infection. Toxoplasmosis is the most widespread zoonosis and an important human disease particularly the congenital infection where it could cause visual and neurological impairment and mental retardation in children. In the present study, the prevalence of *T. gondii* infection in centre of Portugal were determined from January 2000 to December 2006 by testing the sera collected from women (n = 1,003) and men (n = 168) for *T. gondii* immunoglobulins G and M antibodies. The anti-toxoplasma IgM and IgG were determined using ELFA technique (Enzyme Linked Fluorescence Assay) (VIDAS Toxo, BioMériuex). Results for IgG antibodies were reported in International Units (IU); samples with $\geqslant$4 IU were considered positive for *T. gondii* immunoglobulin G antibodies. The results for IgM were reported as positive if the index was >0.65 and negative if was <0.55. In women, the rates of *T. gondii* IgM antibodies were 0.9% (9/1,003) and 24.9% (250/1,003) for IgG antibodies. Along the study period 2000–2006 the toxo anti-IgG ranged from a minimum of 13.6% in 2005 to a maximum of 30.3% in 2001. In men, the rates of IgM antibodies were 0% and 58.9% (99/168) for IgG antibodies. The total rate of positively of *T. gondii* antibodies was 30% (351/1,171) and the negativity was 70% (820/1,171). The age-adjusted *T. gondii* seroprevalence among males with 20–29 years old (n= 24) was 20.8% and among females with 20–29 years old was 27.3% (135/495). The prevalence of *T. gondii* IgG antibodies increased with age: the highest positive rates (83%) have been founded in persons with age more than seventy years old (25/30). These results indicate that preventive measures should be implemented in pregnant women's to prevent the congenital toxoplasmosis.

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P1879 Prevalence of *Toxoplasma gondii* infection in pregnant women in the Albacete area, Spain

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Objective: Determining the seroprevalence of *Toxoplasma gondii* in pregnant women living in the Albacete area, Spain.

Methods: A study on women living in the Albacete area who gave birth during 2006 and had been serologically screened for anti-*T. gondii* IgG – this test is made as a matter of routine to all pregnant women at Albacete general hospital. Their age, place of birth and residence as well as the result of determining anti-*T. gondii* IgG were looked up in the registry.

Results: In 2006, 2,704 births occurred in the study area, 2,627 (97.2%) out of which had been tested in search of anti-*T. gondii* IgG at least once during pregnancy. Four women were excluded from the study as they presented an undetermined result as to anti-*T. gondii* IgG, so that the final total number of women included in the study was 2,623. Prevalence of anti-*T. gondii* IgG amongst studied women was 20.6% (95% CI= 19.1%-22.2%). Seroprevalence amongst Spain-born women was 15.8% (95% CI= 14.3%-17.4%) and 50.8% (95% CI= 45.5%-56.1%) amongst women coming from other countries. Seroprevalence increased linearly with age, both amongst Spain-born ($p < 0.01$; Ji-Square for linear trend) and immigrant ($p < 0.01$; Ji-Squared for linear trend) women. Amongst Spain-born women, seroprevalence increase ranged from 9% (95% CI= 6%-14%) in women under 25 to 22% (95% CI= 19%-26%) in women over 34 years old. Amongst Spain-born women, seroprevalence was lower in those living in the city of Albacete than in those living in cities of less than 30,000 inhabitants (14.1% versus 17.9%; OR= 0.75, 95% CI= 0.59–0.96; $p = 0.016$).

Conclusion: Prevalence of toxoplasmosis in our area is higher amongst immigrant women than in Spain-born women and it increases with age. Amongst those women born in Spain, seroprevalence is lower in women living in the city of Albacete than in those living in smaller cities. Seroprevalence amongst Spain-born women is the lowest one of those published so far. Continuous education of pregnant women about risks of infection is essential.

P1880 Materno-foetal toxoplasmosis: staging of infection and newborn follow-up

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Objectives: *Toxoplasma gondii* infection during pregnancy may be transmitted to the foetus: risk of infection and seriousness of disease depend on gestational age at the time of maternal infection. Aim of this study was to establish correct maternal serologic diagnosis in order to define proper therapy and newborn follow-up.

Methods: During 2004–2006, 33 mother/child pairs were followed up using the following approach: (a) counseling to evaluate fetal risk tied to gestational age at the time of maternal infection, diagnosis of prenatal infection, therapy administration and frequency of ultrasound scans; (b) follow-up of all newborns up to their first year by serologic, immunologic, clinic and instrumental methods. The following tests were used to diagnose maternal infection: *Toxoplasma* IgM, IgG and avidity of IgG by CLIA (DiaSorin, Saluggia, Italy) and detection of *Toxoplasma* DNA in amniotic fluid by nested PCR (Nanogen Advanced Diagnostics, Turin, Italy). The following tests were used for newborn screening: *Toxoplasma* IgM, IgG by CLIA (DiaSorin, Saluggia, Italy), ISAGA (BioMérieux, Marcy l'Etoile, France) and Western blot (LDBIO, Lyon, France). Results were compared with maternal serology outcome.

Results: 33 newborns were followed up by serologic, immunologic, clinic and instrumental methods for one year. Mothers were treated during pregnancy in accordance with routine protocols. Only 5 children were infected, classed as follows: (a) 1 infected child and 13 healthy children in the group of mothers infected during the first trimester of pregnancy; (b) 3 infected children and 15 healthy children in the group of mothers infected during the second trimester of pregnancy; (c) 1 infected child in the group of mothers infected during the third trimester of pregnancy. All infected children were apparently healthy at 1 year of age.

Conclusions: Serologic and immunologic follow-up of children born from mothers infected by *T. gondii* during pregnancy must be extremely accurate, especially during the first 6 months of life. Methods used for antibody detection in mothers and children (ISAGA, CLIA or EIA and WB) are used to anticipate diagnosis of congenital infection, generally obtained for antibody rebound, and to administer proper therapy. In our clinical experience no infected children born from adequately treated mothers showed signs of disease.

P1881 Clinical manifestations of congenital toxoplasmosis and their importance in the prophylaxis of congenital malformations

L.M. Junie on behalf of ESGP

Background: Congenital malformations represent an important aspect of medicine due to its deep social and inside family implications. The toxoplasmic infection is one of the major causes of congenital malformations. We decide to solve some aspects, like the clinical manifestations of congenital Toxoplasmosis.

Material and Method: We studied 253 children with congenital malformation. The *Toxoplasma* infection was detected by demonstrating the presence of specific antibodies by indirect IF and ELISA. The maternal antibodies established in order to identify the acute toxoplasmic infection during pregnancy.

Results: The studied children were newborn(63.8%)or infants(36.2%). Trying to demonstrate the presence of antibodies in 68 pairs of mother-malformed baby, positive results were in 59 cases (86.8%). Our data suggest that 78% of congenital malformations are the consequence of

an infection acquired in the first months of pregnancy, when the fetal embryogenesis take place and only 22% of mothers had the infection during the last period of pregnancy (11.8 in the second semester and 10.3% in the third semester). The evolution of most pregnancies of malformed children mothers was apparently normal (57.3%) and 42.7% had abortion and pathologic pregnancies: stillbirth, haemorrhage, premature birth and others. The majority of malformed children had isolated ophthalmologic (36.6%) and neurological problems (97.6%), cranial malformations (23.8%), visceral disease (12.5%), malformations and visceral disease (11.4%). The neurological manifestations were especially psychomotor retardation (71.3%), epileptic seizures (11%), tetraparesis (2.7%), half paresis (4.2%), cerebellum syndrome (1.4%), cranial nerves paralysis and deafness (7%). As visceral manifestations, we found anaemia (24.7%), thrombocytopenia (1.4%), jaundice (1.4%), and heard malformations (2.7%). The congenital malformations were microcephaly (17.8%), cranial-cerebral dimorphism (9.6%), and hydrocephalus (6.9%), microphtalmy (1.4%), cerebella atrophy (1.4%), frontal-parietal atrophy (1.4%) and cerebral ventricle ecstasy (1.4%).
Conclusions: Our studies afford the necessity of active scientific surveillance of pregnant women and newborn children. They emphasize the need of national programmes for the control of toxoplasmic infections in which modern techniques of diagnosis of antitoxoplasmic antibodies should be used both in pregnant women and in newborn children.

P1882 Comparison of two real-time PCR targets (B1 and REP 529) for *Toxoplasma gondii* DNA detection for the diagnosis of congenital toxoplasmosis

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Objectives: While often asymptomatic, the infection with the protozoan parasite *Toxoplasma gondii* can cause serious damage (i) in the immunocompromised patient and (ii) in the foetus when it is acquired during pregnancy. In both situations, DNA parasite detection is essential for diagnosis, when performed in cerebrospinal fluid (CSF) or blood (in the case of cerebral or disseminated toxoplasmosis), or amniotic fluid (AF) and placenta (for prenatal or neonatal diagnosis of congenital toxoplasmosis, respectively).
Various sequence targets and PCR protocols (conventional PCR, real-time PCR) have been developed for molecular diagnosis of toxoplasmosis.
In this study, we evaluated two real-time PCR assays that we had developed in our lab, using Taqman probes on ABI Prism 7000 system: a PCR assay targeted at the multicopy gene B1 and a PCR assay targeted at the repeated sequence REP-529.
Methods: Both techniques were prospectively or retrospectively applied to 40 clinical samples: 20 samples from patients with proven congenital toxoplasmosis (7 AF, 12 placentas, and 1 cord blood sample), and 20 placentas from women who had seroconverted during pregnancy but whose newborns were later proven non-infected. Serial dilutions of *T. gondii* RH strain were also amplified using B1 and REP-529 target PCRs.
Results: No DNA was amplified with any of the techniques, in the 20 specimens from patients in whom the diagnosis of congenital toxoplasmosis was later ruled out. REP-529 sequence was detected in all 20 samples from patients with congenital toxoplasmosis, whereas B1 gene sequence was detected in only 16 of the 20 specimens. The kappa value (concordance test) was 0.8. False negative results with B1 gene were observed in 3 placentas and 1 AF from foetus that were otherwise proven infected by other techniques and a long-term follow-up of the newborns. Moreover, in the 16 samples that were positive with both target sequences, a gain in sensitivity was obtained with the REP-529 amplification, since it allowed the detection of DNA with a mean Ct value that was 4 cycles lower than the Ct value obtained for B1 amplification. Similar results were obtained with the serial dilutions of the RH strain, used as control.
Conclusion: Real-time PCR using the REP-529 Taqman probes was more efficient than B1 gene amplification for the diagnosis of congenital toxoplasmosis, since it allowed parasite DNA detection in all clinical specimens.

P1883 Development of two combined real-time quantitative OCEANII PCR assays to detect *Toxoplasma gondii* in amniotic fluids and sera

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Objectives: Toxoplasmosis is a worldwide infectious disease caused by the protozoan *Toxoplasma gondii* mainly acquired by feeding raw meat containing tissue cysts. Congenital infected foetuses are at risk for severe or life-threatening disease. In children, the infection may cause cervical lymphadenopathy or ocular diseases with serious clinical course. Early detection of *T. gondii* is therefore crucial to assess proper treatment and to avoid critical sequela. To date laboratories use customised diagnosis methods, which produce a large wide of protocols. Our research aims to provide a real-time quantitative PCR assay for rapid and sensitive detection of *T. gondii* in specimens.
Methods: Two real-time PCR assays, targeting internal portions of the multicopy B1 gene and repeated element RE of *T. gondii*, were selected to design the OCEAN II assays (DiaSorin), based on the specific hybridisation of a fluorogenic primer forming a three-way-junction with an anchor probe. In silico simulations identified optimal paired primers and thermodynamic conditions to run B1 and RE reactions. Ten-fold dilutions from 100,000 to 0.1 copy of RH strain genomic DNA was exploited, in triplicate, to titrate the dynamic range of the real-time PCR and to assess intra- and inter-assay reproducibility in more than ten independent experiments. 1 ml of sera and amniotic fluids, serologically negative for *T. gondii*, were spiked with 10-fold dilutions of tachyzoites from 100,000 to 0.1 in order to quantitatively correlate parasite extracted DNA and genome copies. A group of 25 amniotic fluids and sera, previously tested for *T. gondii* by seroconversion and PCR, were tested, as triplicates, to assay the B1- and RE-based real-time systems.
Results: The linearity of the B1 and RE assays was achieved over seven logs of input DNA, showing a consistent dynamic range and accuracy for genomic DNA in each assay (R[B1] from 0.994 to 0.999, R[RE] from 0.987 to 0.999, respectively). High inter-assay reproducibility was achieved, as inferred by R[B1]=0.998 and R[RE]=0.999. Ct values found with reference and spiked tachyzoite DNA were highly consistent for B1 and RE assays on both sera and amniotic fluids (R[B1]=0.991, R[RE]=0.990) and clinical concordance was obtained for 22 over the 25 collected samples.
Conclusions: The B1 and RE combined real-time PCR assays may represent a new diagnostic tool for rapid and quantitative detection of *T. gondii* in clinical specimens.

P1884 Performance of the Abbott ARCHITECT Toxo IgM assay

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Objectives: Infection by Toxoplasmosis gondii continues to be a ubiquitous disease that may seriously impact the developing foetus. The clinical implications for the child can include neurological defects, blindness and death. Therefore, routine monitoring of the Toxo serological status of pregnant females remains a practice in many countries. The objective of this study was to evaluate the performance of the Abbott ARCHITECT Toxo IgM assay as a means of assisting in the prevention of this disease and the associated congenital defects it causes.
Methods: The Abbott ARCHITECT Toxo IgM immunoassay is based on a Mu- capture format in which the patient's IgM is bound to mouse anti-human monoclonal antibody coated paramagnetic microparticles. Specific detection of the anti-Toxo IgM is acccomplished via incubation with a *Toxoplasma gondii* tachyzoite lysate which is complexed to an acridinium-labeled mouse anti-Toxo P30 monoclonal antibody. Specificity testing included 300 samples from random blood donors, 300

samples from random hospital patients and 299 pregnant female samples from all trimesters. Sensitivity of the assay was determined by testing 42 seroconversion panels and 155 individual Toxo positive samples. The results of this testing were compared to the Abbott AxSYM Toxo IgM assay and employed resolution of discordant samples with another Toxo IgM assay.

Results: Detection of acute infection, as demonstrated with seroconversion panels, was found to be equivalent to that of the Abbott AxSYM Toxo IgM assay. Both detected the same first positive bleed in 40 panels and each was ahead in a panel. Resolved sensitivity for the Toxo positive samples was 99.31% to 100% while that of AxSYM was 96.55. In a comparison to ISAGA IgM assay results, the relative sensitivity of the ARCHITECT assay was 97.33–99.32%. The ARCHITECT Toxo IgM assay was found to have a relative specificity of 100% for the composite population of random blood donors, hospital patients or pregnant females while the specificity for AxSYM Toxo was 99.89%.

Conclusion: The Abbott ARCHITECT Toxo IgM assay demonstrated performance which was comparable to the reference assays and provides an automated method to evaluate the serological status of patients in conjunction with Toxo IgG and Toxo avidity assays.

P1885 Evaluation of qualitative agreement and quantitative correlation between the new ARCHITECT Toxo IgG assay and 4 commercially available Toxo IgG assays

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Objectives: Determination of Toxo IgG antibodies to *Toxoplasma gondii* is mainly important in pregnant women and immunocompromised patients to evaluate their serological status. Commercially available Toxo IgG assays are standardised against International standard preparations and report IU/ml concentrations. However, there were at least three different standard preparations available for standardisation of currently used Toxo IgG immunoassays, which might impact the quantitative correlation and slope between methods.

In this study we describe the qualitative agreement and quantitative correlation of the new ARCHITECT Toxo IgG assay, which is standardised against the WHO First International Standard (01/600) and utilizes recombinant antigens, and 4 other Toxo IgG assays standardised against earlier WHO standard preparations.

Methods: 100 pre-characterized Toxo IgG positive and 100 pre-characterized negative specimens were evaluated on the Vidas, Access, Enzygnost and AxSYM Toxo IgG assays in comparison to ARCHITECT Toxo IgG. Qualitative agreement and quantitative correlation was assessed.

Results: Qualitative agreement between the methods ranged from 98.9 to 100% without grey zone specimens and from 95.5 to 98.4% with grey zone specimens. Slope of linear regression between methods was very variable. The slopes and the correlation coefficient r versus Architect ranged from 0.8 to 3.0 and from 0.81 to 0.96, respectively.

Conclusion: Good qualitative agreement could be observed between all assays evaluated. Nevertheless, the differences in quantitative determination of concentrations were considerable. Quantitative differences can be attributed to differing standardisation, assay format and technology. This underlines the necessity to perform quantitative comparison of Toxo IgG levels within one method only.

P1886 Membrane biogenesis in the intracellular parasite *Toxoplasma gondii* and its significance as a novel chemotherapeutic target

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Objectives: *T. gondii*, an obligate intracellular protozoan parasite, causes severe congenital infections in humans and animals. *Toxoplasma* is also an opportunistic pathogen associated with AIDS. Successful replication of *T. gondii* within its host requires substantial membrane biogenesis. We characterised the phospholipid metabolism of *T. gondii* and the selective disruption of its PtdCho synthesis to inhibit its intracellular growth.

Methods: The phospholipid metabolism of axenic *T. gondii* was investigated using radio-isotopic precursors and thin layer chromatography. The selective inhibition of PtdCho metabolism was achieved by treatment of infected human fibroblasts with dimethylethanolamine (Etn(Me)2).

Results: *T. gondii* can metabolize the lipid precursors, serine, ethanolamine, choline and inositol in a time- and concentration-dependent manner to synthesise PtdSer, PtdEtn, PtdCho and PtdIns, respectively. The parasite lacks the PtdEtn methylation pathway to synthesise PtdCho indicating it is a choline or PtdCho auxotroph. Etn(Me)2 selectively interferes with the PtdCho metabolism of *T. gondii* and causes 99.5% inhibition of its growth within fibroblasts. It is readily incorporated into PtdEtn(Me)2 by the parasite, and during replication within the host cell it becomes the major phospholipid. The synthesis of PtdEtn(Me)2 is accompanied by a decrease in PtdCho. Relative to host cells, the parasites accumulate 7 times as much PtdEtn(Me)2 into their phospholipid, which disrupts the physical and biochemical integrity of parasite membrane as confirmed by electron microscopy.

Conclusions: *T. gondii* has the ability to autonomously synthesise phospholipids, which renders it uniquely susceptible to modifiers of lipid metabolism. Our findings also reveal, how selective inhibition of parasite PtdCho synthesis by a choline analog is a powerful chemotherapeutic approach to arrest parasite growth within its mammalian host.

Virology

P1887 Molecular characterisation of a rare G8P[14] rotavirus strain detected in an infant with gastroenteritis in Italy

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Objective: The aim of the present study was to perform the molecular characterisation and the phylogenetic analysis of a rare G8P[14] group A rotavirus (GARV) strain detected in Parma, Northern Italy, during a 2004–2005 rotavirus (RV) epidemiological surveillance.

Methods: Two hundred and seventy three RV-like particles positive stools by electron microscopy (EM) out of 856 stools from children (median age 1 year 8 months, range 1 month – 10 years 4 months) hospitalised with gastroenteritis in Parma, Northern Italy, were submitted to PAGE analysis.

Genotyping of 271 GARV strains was carried out on dsRNA extracted from 10% PBS stool suspensions by a nested and/or heminested RT-PCR specific for VP7 and VP4 genes, using pools of G and P type specific primers. One strain (PR/1300/04) displayed G8 specificity and was not typeable in the VP4 gene.

After purification by "Qiaquick Gel Extraction Kit" (QIAGEN, Italy), the VP4, VP6, and VP7 first amplicons of the G8 GARV strain were subjected to sequence analysis with automated sequencer 3730 DNA Analyzer (Applied Biosystems, USA). Phylogenetic analysis was conducted using MEGA version 2.1.

Results: The PR/1300/04 strain exhibited P[14] specificity and long e-type. By sequence analysis, the PR/1300/04 VP7 gene displayed high nucleotide (97.9%) and amino acid (99.4%) identities to bovine Japanese and South African strains, while the VP4 gene was closely related (96% and 99.1% nucleotide and amino acid identity) to the human Italian PA169 strain. The VP6 gene was found to contain subgroup I specificity. Phylogenetic analysis demonstrated the animal origin of PR/1300/04 VP7 gene and the human origin of the VP4 gene.

Conclusion: The data obtained seem to suggest that the Italian PR/1300/04 strain could be the result of a reassortment between a PA169-like strain with P[14] specificity, long e-type and subgroup I, already circulating in Italy, and a G8 animal strain. The increasing number of reports of atypical RV strains in humans suggests that interspecies transmission of gene segments greatly contributes to the RV genetic evolution.

P1888 **Genetic characterisation of G3 rotaviruses detected in an Italian infantile population in 1993–2005**

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Objectives: Group A rotavirus are the most common aetiologic agents of acute gastroenteritis in children worldwide. Sequence analysis of the genes coding for the two outer capsid proteins VP7 and VP4, for the inner capsid protein VP6 and for the non structural protein NSP4 is useful to gather epidemiological information on rotaviruses. To date, 15 VP7 G-types, 27 VP4 P-types, 4 VP6 subgroup specificities (SGs I, II, I+II and nonI/nonII) and 5 NSP4 genotypes (A to E) have been established. The most common genotypes are G1P[8], G3P[8] and G4P[8], in association with SGII and NSP4 B, and G2P[4] SGII NSP4 A. Recently, epidemiologic studies showed an increasing detection of G3 strains in Japan, China, Russia, Ireland and Spain. In Italy, in the past G3 rotaviruses have been detected sporadically or at very low prevalence. However, in 2003 and 2005 they acquired an important epidemiological role. To investigate the emergence of G3 rotaviruses in Italy a sequence analysis was performed on G3 strains detected in Palermo during 13 years.
Methods: A total of 1012 rotavirus positive faecal specimens were collected from children (<5 years) hospitalised with acute gastroenteritis at the "G. Di Cristina" Children's Hospital of Palermo, Italy from 1993 to 2005. Rotaviruses were genotyped by nested RT-PCR to define their G and P type. Sequencing and phylogenetic analysis of the VP7, VP4, VP6 and NSP4 genes was performed after amplification with type-specific primers on representative G3 strains.
Results: Fifty-seven (5.6%) rotavirus positive samples were genotyped as G3. Of the 19 strains submitted to sequence analysis 11 were typed as G3P[8] SGII NSP4 B, three as G3P[9] SGII NSP4 A and one as G3P[3] SGI NSP4 C. In the phylogenetic tree of VP7, the Italian G3P[8] and G3P[9] rotaviruses were distributed into lineage I while the G3P[3] in lineage III. Sequence analysis of VP4 revealed that all Italian G3P[8] rotaviruses clustered in the P[8]-3 lineage. Phylogenies of the NSP4 gene showed the clustering of Italian strains in two separate groups according to the year of sampling.
Conclusions: The genetic analysis performed ascertained that strains circulating in Palermo shared common features in the genome segments analysed but amino acids mutations in the NSP4 sequence appeared in 2003 and were conserved through 2005. The periodical increase of G3 strains circulation seems more linked to a loss of immunity in the population that to changes in their antigenic determinants.

P1889 **Electrophoretic RNA genomic profiles of rotavirus strains prevailing among hospitalised children with acute gastroenteritis in Tehran, Iran**

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Background: Human Rotavirus (HRV) has now been established as the most important cause of childhood gastroenteritis worldwide. The RNA genomic electrophoretic diversity of group A Rotavirus strains in Tehran, Iran, Between April 2002 and March 2005, were investigated in children stool specimens.
Methods: A total of 1250 Stool samples were obtained from hospitalised children younger than 5 years old with acute gastroenteritis and RNA electrophoretype of group A Rotavirus strains was determined using RNA-PAGE in those samples found to be positive for Rotavirus by EIA.
Results: The predominant RNA profile detected was the long electrophoretype (90%) followed by the short profile (8.8%). Four cases (1.2%) had patterns of mixed infection. Fourteen different electrophoretic RNA patterns, eight of long and six of short, were detected in the study area. A predominant long RNA electrophoretype persisted during the years study with peak incidence in cool seasons of the year. The long pattern occurred throughout the study period and in all age ranges of patients (1–60 months), but the short profile identified only in infants (1–24 months) at varying intervals.
Conclusions: Our data demonstrate epidemiologic differences between long and short electrophoretypes incidence; the long profiles appear to circulate continuously in the area, whereas the short electrophoretypes appear in an episodic fashion. This study highlights the simultaneous co-circulation of multiple group A Rotavirus strains in Tehran and stresses the need constant monitoring of the genomic diversity resulting from extensive genomic variation among Rotaviruses in the area.

P1890 **Rotavirus diversity in Bulgaria during 2007**

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Background: Rotaviruses remain one of the leading causes of morbidity and mortality worldwide. Rotaviruses are RNA containing viruses with segmented genome and their strain diversity and intensive evolution are based on accumulation of point mutations and reassortment events. Rotaviruses are highly infectious and easily distributed among human population which are preconditions for appearance of new endemic/epidemic strains. Objectives of the stidy were investigation of prevalence and rotavirus strain diversity in Bulgaria during 2007 and comparision of data obtained with the data from previous years.
Materials and Methods: A total of 1267 stool specimens collected from January to mid October 2007 from children under 10 years of age with acute gastroenteritis were screened for rotaviruses by enzime immunoassay, confirmed with PAGE and G and P genotyped by RT-PCR using two sets of primers.
Results: The results revealed: 1) 29.4% (373 of 1267) stools were rotavirus-positive; 2) the most predominant G and P genotypes detected were G2 (52.8%, 105/199) and G9 (36.2%, 72/199) and P[4] (44.7%, 89/199) and P[8] (28.6%, 57/199) respectively; 3) 34.7% (69/199) of rotavirus strains failed to be fully/partly genotyped by Das-Cunliffe/Gentsch primers and 59 strains were retested using Gouvea/Iturriza primers set; 4) 71.2% (42/59) of rotaviruses remained G and/or P-untypeable using two primers sets and ongoing sequence analysis will uncover the probable resons for this; 5) "shift" in the incidence of rotavirus genotypes: G4P[8] in 2005, G9P[8] in 2006 and G2P[4] and G9P[8] in 2007; 6) significant incidence of infections with G2P[4] among older children aged 2–10 years; 7) year-round rotavirus circulation with a peak in winter season and appearance of the second peak in August-September.
Conclusions: Broad and long-term rotavirus detection and characterisation among children aged 1 month-15 years using new and improved detection assays and primers sets will describe the epidemiology of rotavirus infections in Bulgaria and driving forces of possible appearance of new rotavirus strains.

P1891 **Aetiology of paediatric viral gastroenteritis in Bulgaria**

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Background: Acute gastroenteritis is one of the most prevalent diseases worldwide, being among the leading causes of morbidity and mortality in infants and young children. The majority of enteric infections are viral in origin. The main enteric pathogens are members of four families – rotaviruses, noroviruses, astroviruses and enteric adenoviruses. The objective of the study is to determine the distribution of viral agents in hospitalised children with acute gastroenteritis.
Methods: From December 2006 to April 2007 a total of 470 stool samples from paediatric patients exhibiting symptoms of diarrhoea and/or vomiting were tested for the presence of enteric viral pathogens using immunoassay kits (Ridascreen™, R-Biopharm). The results from ELISA method were compared with polyacrylamide gel electrophoresis (PAGE) for rotaviruses. RT-PCR was used to confirm rotavirus and norovirus antigen-positive results and to characterize strain specificity.

Results: Viral pathogens were identified in 215 (45.7%) samples. Group A rotaviruses were detected in 136 (28.9%) followed by noroviruses – 52 (11.1%), enteric adenoviruses – 36 (7.7%) and astroviruses – 1 (0.2%). Mixed infections were proved in 10 (4.7%) cases. The most common co-infections were observed between rotaviruses and adenoviruses. Using RT-PCR techniques two dominant rotavirus strains (G2P[4] and G9P[8]) and GGII.4 norovirus strains were identified and confirmed by sequencing. The majority of cases were in children aged 7–36 months and predominated between February and April.
Conclusions: The results of the first investigation of causative agents of acute gastroenteritis in hospitalised children highlight the important aetiologic role of viral diarrhoeal pathogens in Bulgaria and the significance of rapid screening methods for achieving an early diagnosis.

P1892 Ten-years surveillance of norovirus in outbreaks of gastroenteritis in Hungary: molecular epidemiology, genetic diversity and evolution

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Objectives: Noroviruses are important pathogens in outbreaks of gastroenteritis in humans wordwide and they belong to a genetically diverse group of RNA viruses (family Caliciviridae) with multiple genogroups (G) and genotypes.
Methods: Between November 1998 and June 2007, 735 (44.6%) of 1647 reported outbreaks of gastroenteritis were investigated prospectively for norovirus in Hungary. Stool samples in outbreaks of nonbacterial gastroenteritis were examined by ELISA and RT-PCR methods.
Results: Norovirus was detected in 724 (98.5%) of 735 outbreaks and confirmed by sequencing in 500 (69%) of epidemics. Strains grouped into 2 genogroups (GI, 4.6%; GII, 95.4%) and 15 genotypes (GI-1, 2, 3, 4, 6 and a new genotype; GII-1, 1b, 2, 5, 7, 8, b, and a new genotype) with the predominance of GII-4-Lordsdale (76%) genotype. Four main genetic variants (epidemic point-mutants) of GII-4 were identified and they appeared subsequently in every 2 years (GII-4–2000; GII-4–2002; GII-4–2004 and GII-4–2006b). Each of them associated with strong, 2-years long epidemic periods mainly in healthcare institutions and elderly homes. In 2006, an unusual summer and even the highest winter activity were detected associated with GII-4–2006b. This variant was also identified in the largest waterborne outbreak ever seen in Hungary and affected more than 3600 patients in June 2006. In this outbreak, the public water system of a large town supplied by karstic water was contaminated as a consequence of extremely heavy rainfalls flooding the karstic area. Since 2000, continuous circulation of recombinant strain GIIb with 4 capsid types were also detected as a second most common (11%) genotype associated with outbreaks in children. Nearly 90% of the norovirus outbreaks was caused by only 2 genetic types, the point mutant variants GII-4 and the genetic recombinant GIIb.
Conclusions: According to this 10-years study noroviruses were the predominant aetiological agent causing gastroenteritis outbreaks in Hungary. Elevated numbers of norovirus outbreaks in the population most likely indicate the emergence of a new GII-4 drift variant as part of an international epidemic/pandemic. Genetic drift successfully promotes the re-emergence of genotype GII-4 in the population. This phenomenon is highly similar to influenza virus antigen drifts when sufficient level of immunity in the population has been developing against one predominant strain over nearly a period of every 2–3 years.

P1893 Retrospective comparison of human papillomavirus detection in 42 HSIL samples, by using RNA-based NucliSENS EasyQ™ HPV Assay and DNA genotyping

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Objectives: The aim of this study was to evaluate the new NucliSENS EasyQ™ HPV assay (bioMérieux, Boxtel, The Netherlands) by using the NucliSENS easyMAG® and NucliSENS EasyQ® systems in comparison with cytology, histology and DNA genotyping.
Methods: Retrospective cervical HSIL samples from 42 women 22–50 years of age, collected between August 2006 and August 2007 and stored at room temperature in PreservCyt® transport medium (Cytyc Corp, MA, USA) were analysed in October 2007.
Total RNA was extracted on NucliSENS easyMAG® system from 1 ml cervix sample and eluted in final volume of 50 μl. Eluates were used for both mRNA and DNA amplification.
E6/E7 mRNA expression from high-risk HPV 16, 18, 31, 33 and 45 was detected with multiplex NASBA (NucliSENS EasyQ™ HPV) and compared with DNA typing results. HPV DNA genotyping was carried out with INNO-LiPA HPV Genotyping (Innogenetics, Ghent, Belgium), according to the manufacturer's procedure.
Results: All samples (42) were defined by pathologist as high-grade squamous intraepithelial lesion (HSIL). Thirty-five samples of CIN2+ grade and 2 samples of LSIL were fully concordant with cytological and histological results. The 5 other samples were not histologically-evaluated.
Internal control mRNA was successfully amplified in all cases suggesting no inhibition or degradation. Among 42 analysed samples, 38 were HPV-positive (90.5%). Their mRNA based genotypes were: HPV-16 (68%), HPV-18(8%), HPV-31(8%), HPV-33(13%) and one multi-infection HPV-16/31 (3%). All these genotypes but one (HPV-33 vs HPV-58) were found identical by DNA genotyping.
From the 4 HPV-negative mRNA samples, 3 were determined by DNA genotyping as positive for HPV-51 or HPV-58 type and one HPV-16. The latter could not be tested further for mRNA.
Conclusion: In the present study, NucliSENS EasyQ™ HPV assay, designed for the detection of the 5 HPV types 16, 18, 31, 33 and 45, have shown a good correlation with histology.

P1894 HPV-E5 oncoprotein downregulates IFN-beta signalling in HaCaT/E5 cells

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Objectives: HPV oncoprotein are known to interfere at several levels with both innate and adaptive responses of the host. While the role of the oncoprotein E6 and E7 is very well known, the biological functions of E5 are poorly understood. E5 is expressed early in papillomavirus infection, co-operates with epidermal growth factor, prevents transport of the major histocompatibility class I to the cell surface and retains the complex in the Golgi apparatus. Since the E5 functions seem to be linked to virus ability to circumvent host immunosurveillance, we focus our attention on the role of E5 protein in the interferon signalling pathway and we investigated the expression of signal transducer and activator of transcription (STAT) protein and the expression of some IFN-stimulated genes (ISGs).
Methods: We used Western Blot to investigate the amount and the phosphorylation of STAT-1 in HaCaT/E5 cells expressing only E5 gene of HPV and HaCaT/pMSG as control. Moreover, we used RT-PCR to address the expression of ISGs including PKR, LMP2, IL-12 and Bak, which are involved in host resistance to infection and immune function.
Results: We found inhibition of STAT-1 amount and phosphorylation in cells expressing E5 gene. The E5 protein in turn exerted a transcriptional repression on several interferon-stimulated genes, including those codifying for IL-12, PKR, LMP2 and Bak.
Discussion: These data described pathogenetic effect of HPV-E5 protein and suggest that E5-induced STAT-1 modulation may play a pivotal role in establishing persistent infection.

P1895 Clinical utility of low-density microarray to evaluate human papilloma virus genotype distribution in Danish women with atypical cervical cell findings diagnosed with HPV infection

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Objectives: Use of microarray technology (MA) in clinical Human Papilloma Virus (HPV) diagnostics offers simultaneous diagnostic

capability along with genotyping of specific subtypes. Here, we report on the use of HPV DNA MA analysis to perform detailed diagnostics and genotyping of HPV infections in women with atypical cervical smears.

Methods: HPV-DNA microarray analysis were performed on 374 individual, clinical sam-ples from women with atypical cervical smears diagnosed with HPV infection. The low-density HPV MA system (Genomica, Spain) contains specific probes for 35 mucosal HPV types, commonly associated with a higher risk of cervical dysplasia or cervical cancer.

Results: Use of MA technology allows for simultaneous detection and genotyping of mul-tiple HPV types. 41% of the individual samples were found positive for one HPV geno-type, while 27% were positive for 2 HPV genotypes, 12% were positive for 3 HPV geno-types, and 11% were postive for 4 or more simultaneous HPV genotypes. Up to 12 individual HPV genotypes were detected in a single sample. Women <25 years had the higest incidence of multiple HPV infections (63.3%), whereas 37% of the women age >45 years were found to have >2 simultaneous HPV genotypes. Data from HPV postive samples have resulted in a newly ranked list of HPV genotypes associated with atypical cervical cell findings in Danish women: HPV 16 (13.8%), was most commonly found whereas HPV 72 (0.35%) was rarely found. A complete list ranking all 35 types included in the analysis will be presented. An analysis of HPV co-infection patterns was subsequently performed. HPV type 16 was most commonly found as a single infection (24% multiple infections versus 76% single infections), whereas HPV type 11 was predominantly found as part of multiple infections (82% multiple infections versus 17% single infections). Co-infection patterns for all 35 analysed HPV genotypes will be presented. Finally, the MA analysis was used to perform longitudinal follow up on individual patients where >3 consecutive samples over >2 years were available. Data from these samples showed that the MA approach allowed for better diagnostics of HPV types causing chronical infections as compared to a standard My9/11 PCR-Sequencing diagnostic analysis.

Conclusion: Use of HPV-DNA MA technology greatly improves the diagnostic power with regard to determining single as well as multiple HPV infections associated with atypical cervical cell findings.

P1896 Point prevalence study of HPV in a high-risk population in Havana, Cuba

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Objectives: To estimate the human papillomavirus (HPV) prevalence, classify the infection according to existing genotype risk groups and estimate its association with previous diagnosis of cervical dysplasia in an urban, high risk population, in Havana City, Cuba.

Methods: A random, point prevalence study in a population with a high risk for STD's in urban areas of Havana City, Cuba. 375 healthy young adults, 294 females and 81 males, with an age range from 14 to 35 years old gave consent to participate and been tested for STD's. A cervical smear in women and urethral smear in men was used for molecular detection and genotype risk group classification of HPV by means of multiplex HPV PCR and subsequent microarray analysis.

Results: Prevalence of HPV was 45.6%(CI; 40.4–50.8), affecting particularly females (42.1%; 158/294; OR: 6.1:CI; 37.0–47.3) between age groups 15 to 20 and 21 to 25 years (14.4%;54/97, respectively). In general, well known high risk genotypes were present in more than half of the positives (53.2%;91/171), but infection with types of unknown risk represented an important part of the sample (33%; 57/171). Nevertheless, most common low risk genotypes (70, 42, 61) and unknown risk genotypes (2, 3, 57, 74, 87, 71, 84) were usually co-infecting patients with high risk genotypes. Co-existing HPV infections of genotypes of the same risk group and different risk groups, resulted in only 44.4%(76/171) of cases with single infection. Major associations were seen among genotypes 71 and 2, 42 and 57, 2 and 3, 2 and 84, 3 and 71, 3 and 84, 39 and 2, 45 and 70, 70 and 2, 70 and 68, 84 and 71, 84 and 28. HPV was detected in 8 out of 16 females with a previous history of cervical dysplasia, among them only one carrying a low risk genotype (6.3%; 1/16).

Conclusion: HPV is highly prevalent among the studied population with genotypes 45, 16, 18 the most common high risk genotypes found. Overall, a broad spectrum of HPV types was found and in almost half of the infected individuals more than one HPV genotype was detected. Multiple infections are a known risk factor for the development of cervical cancer. Although the relation between current detection of HPV and history of cervical dysplasia could not be completely clarified in this study, genotypes of unknown risk were found in half of these participants.

P1897 HPV genotypes in anal samples from Italian and foreign-born males attending an STD unit in Milan, Italy

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Objective: Anal intraepithelial neoplasia (AIN) arising from oncogenic HPV genotypes is increasingly reported, especially in MSM, and is less characterised than cervical disease. The relative distribution and pathogenicity of HPV types has been reported to vary by geographical region arising some questions on the relative efficacy of preventive HPV vaccines. This study wants to evaluate HPV genotypes spectrum in a cohort of males attending an STD unit in MIlan, Italy according to their nationality.

Methods: Anal Pap smear, Hybrid-capture-II HPV test (HC2) and HPV genotyping by RFLP technique performed in Italian (IT) and foreign borne (FB) males recruited from Jan 2003 to Dec 2006 in the STD unit of the II Dept of Infectious Diseases, L Sacco Hospital, Milan (Italy) have been evaluated.

Results: Among 194 IT and 47 FB males recruited, HIV infected patients were 165 (85.05%) and 40 (85.1%) respectively. FB people was younger than Italian (mean age 32.1±6.1 vs 37.4±9.7 p 0.0003) and were mostly from south America (91.5%).

Mean CD4 cells and HIV RNA in HIV infected persons at time of evaluation were comparable. FB patients had a higher rate of abnormal results (ASCUS, LSIL or HSIL) on anal cytology (93.2% vs 80.9% p 0.046). Distribution of High Risk (HR) HPV genotypes on HC2 was comparable as so as results on histology.

HPV-16 was identified in 41.2% of IT and 47.5% of FB and constitutes the 22.8% and 22.6% of all isolates in the 2 groups respectively; HPV type specific prevalence was different between IT and FB people for most of the 29 other different HPV genotypes identified. HPV-13, -40, -44, -54, -64, were specifically isolated among Italian while HPV-45, -66, and -69 only in FB persons. Multiple HPV genotypes infections were demonstrated in 51.7% of Italian and in 68.3% of FB people (p = NS) with a higher prevalence of HR-HPV other than 16/18 in this latter group (26.1% vs 42.5% – p 0.041).

Discussion: As already reported for cervical disease, HPV-16 is by far the most common genotype found in anal cytological samples independently from geographycal region while the overall distribution of other HPV genotypes is not overlapping in Italian and Foreign Borne people. These last patients were more likely to be infected with HR-non HPV16/18 types and to harbour more than 1 genotype. The influence of each genotype and the association with the most prevalent HR HPVs on cancer development need to be tested.

P1898 Ribavirin and peg-interferon with or without adjuvant therapy with erythropoietin in patients with hepatitis C: a randomised controlled trial

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Background: It has been established that dose reduction of Ribavirin and interferon leads to reduction in end of treatment and sustained virological response. Erythropoietin can be used to minimise the dose reduction. However, its effect has not been investigated in the randomised controlled trial yet. Thereby, our objective was to assess the effect of erythropoietin

therapy on the Ribavarin and Peg interferon dose reduction in patients with hepatitis C.
Methods: Ninety-four patients with hepatitis C who received Ribavarin plus Peg interferon therapy were randomised to erythropoietin (n = 46) or no erythropoietin (n = 48) adjuvant therapy for 3 months. All patients received 800 mg daily and Pegasys 180 ucg weekly. Patients in the experimental group received erythropoietin when dose reduction was indicated (HGB < 9). The dose of Ribavirin and Pegasys in the control group was decreased when HGB < 9 and these patients did not receive adjuvant therapy at all. T-test was used to compare end of treatment response and sustained virological response between two groups. P-value less than 0.05 was considered as significant.
Results: Both groups were comparable at the baseline. End of treatment response in the experimental group was 91% (42 of 46 patients) versus 69% (33 of 48 patients) in the control group (p = 0.01). Sustained virological response was 87% (40 of 46 patients) in the intervention group as compared with 65% (31 of 48 patients) in the control group (p < 0.01).
Conclusion: Adjuvant therapy with erythropoietin over no erythropoietin therapy results in more favourable outcomes in patients with hepatitis C.

P1899 Anti-orthopoxviral potency of small interfering RNA targeting two genes of the vaccinia virus replication complex

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Objectives: The concerns about the potential use of smallpox in bioterrorism have stimulated the development of novel antiviral treatments. Currently, there are no FDA-approved therapies against smallpox and new treatment strategies are needed. In this study, specifically designed small interfering RNAs (siRNAs), targeting two proteins, i.e., D5R and B1R, essential for orthopoxvirus replication, were investigated for their ability to inhibit vaccinia virus replication.
Methods: Cultures of A549 cells were infected with vaccinia virus strain Western Reserve (VACV-WR), cowpox virus or monkeypox virus, at a multiplicity of infection of 0.1. Transfections with siD5R-2 and siB1R-2, i.e., siRNAs targeting the D5R and B1R genes respectively, were performed for one day, before or after VACV-WR infection, at concentrations ranging from 1 to 100 nM. VACV-WR virus yield production was determined and total mRNAs were extracted for real-time RT-PCR.
Results: siD5R-2 and siB1R-2 decreased VACV-WR replication by 90% when used either prophylactically or therapeutically (P values <0.05). Both siRNAs induced a striking concentration-dependent inhibition of VACV-WR replication and a prolonged prophylactic antiviral effect that lasted for 72 h. They did not induce any interferon-beta production, indicating their specific antiviral potencies. Moreover, our results of real-time RT-PCR demonstrated the specific reduction of the D5R and B1R steady state mRNA levels under siD5R-2 and siB1R-2 treatment, respectively. These data are in agreement with the inhibition of D5 protein expression as determined by western blotting analysis. Confocal microscopy examinations showed the efficient siD5R-2 transfection in the cytoplasm of the cells (using a fluorescent siD5R-2) and revealed a decrease of green fluorescence emitted by VACV-WR labelled with an anti-VACV FITC antibody. We also demonstrated the antiviral potency of those two siRNAs against a pathogenic orthopoxvirus important for human health, monkeypox virus. Synergistic effects between different siRNAs, as well as in vivo studies using mice models are currently under investigation.
Conclusion: Our findings demonstrate the anti-orthopoxvirus potency of siD5R-2 and siB1R-2 and suggested their use as potential candidates to treat poxvirus infections.

P1900 Antiviral activity of isoxazolecarbonitriles against Coxsackievirus A9

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Objectives: Coxsackievirus A9 (CAV9) is a human pathogen causing symptoms ranging from common colds to fatal infections of the central nervous system.
In previous studies, it was found that 5-aryl-3-methylthio-4-isoxazolecarbonitrile derivatives (USA Patent: 6,414,004) possessed a promising antipicornavirus activity. In view of the novelty of this structural class as an anti-picornavirus agent, the in vitro antiviral activity of these compounds against the CAV9 replication was studied. Moreover, we set up some experiments in order to provide information about their mechanism of action.
Methods: The inhibitory effect of 5-aryl-3-methylthio-4-isoxazolecarbonitrile derivatives was studied by plaque reduction assay in RD cell culture.
In order to study the mode of action, we carried out a series of experiments, including virucidal, pre-treatment, inhibition of virus adsorption and time of addition assays.
Results: The new series of isoxazole derivatives were found to have an inhibitory effect on CAV9 replication at doses below the cytotoxic dose. Our results suggest that the antiviral activity was dependent on the nature of the substituents on the para position of the phenyl ring. In fact, compounds with smaller groups on the para position of the phenyl ring (–OCH3 and –OH for compounds ON-0 and ON-3, respectively) were completely ineffective against CAV9.
Compound ON-2, with a –OBn group on the para position of the phenyl ring, showed a slightly antiviral activity.
Isoxazole derivatives (ON-6, ON-7, ON-7/1, ON-7/2) with a longer intermediate alkyl chain between the phenoxy and the phenyl rings exhibited an interesting anti-CAV9 activity with high selectivity indexes.
No of the tested compounds showed virucidal activity nor any protective action for the RD cells.
The effect of the most active compound (ON-7/1) on different steps of the replicative cycle of CAV9 replication revealed that this was significantly inhibited only if the compound was added within 2 h of infection, indicating an interference with an early step of the viral replicative cycle of the virus.
The influence of the compound on the virus adsorption step, studied by the infective centre assay, indicated that ON-7/1 did not interfere with cellular attachment of virus.
Conclusion: These data suggest that ON-7/1 interferes with an early event of Coxsackievirus A9 replication, after viral adsorption. Further studies are necessary to understand the precise mode of action of this compound.

P1901 Identification of a novel group 1 coronavirus from Chinese horseshoe bats

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Objectives: We conducted an extensive surveillance for coronaviruses in Chinese horseshoe bats in Hong Kong and Guangdong province of Southern China, in order to better understand the epidemiology and evolution of the novel group 1 coronavirus and explore possible recombination events between this coronavirus and bat-SARS-CoV that could have led to the emergence of SARS-CoV.
Methods: The respiratory and alimentary specimens of Chinese horseshoe bats (Rhinolophus sinicus) were collected and subjected to RNA extraction and RT-PCR for coronaviruses. Those positive samples were sequenced and analysed by comparing with the sequences of the pol genes of known coronaviruses in the GenBank database. Four complete genomes of bat-CoV detected in the present study were amplified and sequenced.

Results: A total of 770 respiratory and alimentary specimens from 348 and 64 Chinese horseshoe bats were obtained in Hong Kong and in the Guangdong province in Southern China, respectively. RT-PCR for a 440-bp fragment in the RdRp genes of coronaviruses was positive in alimentary specimens from 58 (16.7%) of the 348 bats from Hong Kong, and from 8 (12.5%) of the 64 bats from Guandong. None of the respiratory specimens was positive. Sequencing results suggested the presence of two different coronaviruses among the 64 positive bats. Complete genome sequencing of four strains of bat-CoV revealed the smallest coronavirus genome (27164 nucleotides) and a unique spike protein evolutionarily distinct from the rest of the genome. This spike protein, sharing similar deletions with other group 2 coronaviruses in its C-terminus, also contained a 15-amino acid peptide homologous to a corresponding peptide within the RBM of spike protein of SARS-CoV, which was absent in other coronaviruses except bat-SARS-CoV. Further studies are required to determine if inter-group gene exchange was responsible for the origin of the spike of bat-CoV and its possible role in the evolution of the spike of SARS-CoV.
Conclusion: This is the first report that documents the presence of a spike evolutionarily distinct from the other parts of the coronavirus genome. It also suggests that the RBM of SARS-CoV and the corresponding region in bat-CoV and bat-SARS-CoV share different degrees of homologies, consistent with the hypothesis that the RBM of SARS-CoV may have originated from recombination events.

P1902 Toscana virus, a "Mediterranean" agent of aseptic meningitis: analysis of 42 consecutive cases

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Objectives: Aseptic meningitis (AM) is mostly caused by a number of viruses; among those, Toscana virus (Tosv) is an important arbovirus that is endemically present in our region as well as in both South-European and North-African shores of the Mediterranean basin. Here we describe the main features of 42 cases of Tosv-related AM observed in our unit during a seven-year period.
Methods: Between June 2000 and December 2006, a total of 94 patients with a diagnosis of AM were admitted to our unit. Of those, 42 cases (44.7%) resulted positive for IgM + IgG anti-Tosv as detected by an immune-capture test using monoclonal antibodies bound to the solid phase (IgM) and by an ELISA technique (IgG) (Chorus®, Diesse, Italy). Demographics, clinical picture, neuroimaging, and cerebrospinal fluid (CSF) findings of Tosv-related AM were reported.
Results: In our area, Tosv-related AM was prevalent during summer (73.7%) and by 30 years of age (52.4%) without differences per gender (male 54.8%, female 45.2%). Only 31.1% of patients habitually lived in the urban area of Florence, the remainder residing in flat and hilly surroundings. Clinical course was always self-limited, with a mean duration of the hospital stay of 6.2 days. Headache was present in all cases; it had an abrupt onset, and was mostly diffuse and moderate-to-severe. Clinical picture typically included nuchal/spinal rigidity (85.7%), moderate fever (78.6%), vomiting (64.3%), photophobia (35.7%), and nausea (33.3%). Systemic manifestations such as asthenia, joint and muscle pain, diarrhoea, abdominal discomfort, and pharyngodinia as well as impairment of consciousness were infrequent. Electroencephalography and CT scan showed brain abnormalities that were typical of encephalitis in four patients and in another one, respectively. Chemical analysis revealed clear CSF with increased levels of proteins and white blood cells (mostly mononuclear) and normal levels of glucose and chloride in all cases. No specific therapy was required.
Conclusion: As in other surveys, prevalently from Italy, our patients with Tosv-related AM generally showed clinical, radiological, and laboratory features of a self-limited disease. It is important that clinicians are universally familiar with this infection, that could be acquired during stay in endemic areas of the Mediterranean basin (Portugal, Spain, Southern France, Italy, Cyprus, North-African countries) and then clinically revealed in North America and North-Central Europe, where it is little or nothing known.

P1903 Molecular typing and epidemiology of Enterovirus in Belgium, 2005–2007

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Objectives: Non-Polio Enteroviruses (EVs) are the most common cause of aseptic meningitis. To evaluate the epidemiology of EVs in Belgium during the last 3 years, molecular typing of EV positive in cell cultures and in cerebrospinal fluid (CSF) samples was developed, based on RT-nested PCR and sequencing analysis.
Methods: From January 2005 to August 2007, typing of EV was carried out by immunofluorescence using monoclonal antibodies on viral cultures or genotyping. Eighty-seven cultures, grown on MRC5 or Vero cells, were identified as EV positive. During the same period, 121 CSF from patients suspected of aseptic meningitis were found to contain EV RNA using PCR or nested PCR assays targeting both 5′UTR and VP1 regions with combinations of PCR primers described previously (Oberste et al, J.Virol. 1999; Ishiko et al, JID 2002; Thoelen et al, JCM 2004). In addition, sequencing of 5′UTR or VP1 regions allowed the identification of EV type by comparison with the sequences available in Genbank and by alignments in the Bionumerics software (Applied Maths, Belgium).
Results: As expected, culture yield for EV isolation on CSF was poor (26%). All positive cultures, typable or not with monoclonal antibodies, were identified by sequence analysis. One hundred twenty-one CSF samples could be genotyped by our sequence analysis protocol. For 2005, the major genotyped EVs were Echovirus 9, Echovirus 13, Echovirus 6 and Echovirus 18 (24%, 22%, 14% and 14% respectively). For 2006, the predominant EVs were Echovirus 6, Coxsackie type B3 and B5 and Echovirus 18 (22%, 14%, 14% and 12% respectively). Finally, from January to august 2007, the principal types were Echovirus 30, echovirus 11 and echovirus 13 (with 40%, 16% and 12%, respectively).
Conclusion: In Belgium, during those 3 years, the data's showed that EVs were mostly belonging to group B but with a diversity of genotypes. However, three genotypes (Echovirus 6, 9 and 30) were involved in aseptic meningitis each year of this period, with a clear success of Echovirus 30 during 2007 (40% of cases). The genotyping protocol is rapid, sensitive and accurate for molecular typing of EVs directly on CSF samples and is very useful for epidemiological studies.

P1904 Epstein-Barr virus genotypes and LMP-1 gene variants in paediatric liver transplant recipients

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Objectives: The two Epstein-Barr virus (EBV) genotypes vary in their geographic distribution, tissue tropism and biological features. Latent Membrane Protein 1 (LMP1) is an oncogene and it is expressed in most EBV-positive post-transplant lymphoproliterative disorders (PTLD). Previous studies showed that 30- and 69 bp-deleted variants of LMP-1 have higher transforming capacity. The aim of this study was to determine the prevalence of A and B type and LMP-1 gene variants in blood and tissues of children after liver transplantation (LTx) including PTLD patients.
Methods: 115 children who had undergone LTx at Childrens' Memorial Health Institute between 1999 and 2007 were included in this study. The study involved 13 patients with histologically confirmed PTLD. Molecular assays were performed in blood samples of all children, and in 38 paraffin-embedded tissues from 24 patients. EBV genotype was determined by PCR by simultaneous analysis of two gene loci, EBNA-2 and EBNA-3C. LMP-1 variants were detected by PCR using primers flanking the site of characteristic 30- and 69-bp deletion.
Results: EBV genotype was determined in blood samples from 69 pts and in tissues from 16 pts (including 11 PTLD pts). Type A was detected in blood of 66 (95.6%) pts (including 11 PTLD pts) and in tissues of 15 pts (including 7 PTLD pts). Type B was detected in blood of 3 (4.4%) pts (including 1 PTLD pt) and in 2 tissue samples from one PTLD pt. Interestingly, coinfection with A and B EBV genotypes with different

tissue distribution (liver and lymph node respectively) was determined for that PTLD patient.
LMP-1 was successfully amplified in blood samples from 65 pts (including 12 PTLD pts) and in tissues from 16 pts (including 11 PTLD pts). 30-pb deletion variant was found in blood of 11 (16.9%) pts (including 2 PTLD pts) and in tissues from 3 (10.7%) pts (1 PTLD pt). Both wild type and 30-bp deletion variant were detected in blood of 9 (13.8%) pts and in tissues from 3 (27.3%) PTLD pts. No patient had a 69-bp LMP-1 deletion variant detected.
Conclusion: The strain A with wild type LMP-1 gene is the most prevalent in children after LTx. The 30-bp deletion in LMP-1 gene does not seem to be associated with higher risk of PTLD development. There is a possibility of dual infection either with different EBV genotypes or LMP-1 gene variants, which can show different tissue distribution.

P1905 **Molecular epidemiology of hepatitis C virus in Romania**

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Objectives: To evaluate a home-made genotyping method for investigation of Hepatitis C virus (HCV) variability and tracing infections in Romania.
Methods: sera from Romanian patients were analysed by RT-PCR and sequencing in the core region. PCR amplicons with degenerated primers were sequenced using an automated sequencer ABI Prism 3100 Avant (Applied Biosystems). Multiple sequence alignments and translation into amino acid sequences were performed with BioEdit programme. NCBI BLAST database was screened for similar sequences. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 4.0 with the neighbour-joining method. The distance matrix was calculated by the Kimura two-parameter method using DNADIST.
Results: Two hundred sera from Romanian patients were analysed in the core region using a home-made semi-nested PCR and sequencing. Genotype distribution was: 1b – 95%, 1a – 3%, 4a – 1.5% and 3a – 0.5%. Within subtype 1b, the Romanian strains were intermixed with other 1b strains circulating in European countries. Genetic distances between six 1a strains isolated from injecting drug users revealed a single source of infection. The 4a strains presented 97% homology with the strain ED43 from Egypt. All the core sequences obtained presented an alternative core reading frame. A nosocomial outbreak of HCV infection in a urology clinic from Cluj was investigated using the core sequence analysis. The analysis of 300 nucleotides in the core region allowed identification of a transmission event among the medical staff, involving one source and two infected persons. The core sequences from three patients were monophyletic in the core phylogenetic tree and presented genetic distances of 0.004–0.007 (Kimura two-parameter method). The other unrelated 1b strains presented genetic distances of 0.040 from the outbreak strains.
Conclusion: The new RT-PCR in the core region has a good resolving power for HCV subtyping and evaluating transmission routes. The predominant HCV subtype in Romania is 1b, while subtypes 1a and 3a might have been recently introduced in Romania as a result of drug abuse in the younger population.

Therapeutics and infection in animal models

P1906 **Comparison of tigecycline and vancomycin for treatment of experimental foreign body infection due to meticillin-resistant *Staphylococcus aureus***

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Objective: The therapeutic activity of tigecycline (TIG), which has a broad-spectrum activity against multi-resistant organisms including MRSA, was compared to that of vancomycin (VAN), in a subcutaneous tissue cage (TC) rat model of chronic, foreign body-associated MRSA infection. Previous studies with this animal model have demonstrated the necessity to reach high local levels of bactericidal antibiotics to decrease CFU counts in MRSA-infected TC.
Methods: Strain MRGR3, a highly virulent MRSA isolated from catheter-related sepsis, was used. Freshly prepared, cation-adjusted Mueller-Hinton broth (CAMHB) was used for each MIC macrodilution assay. Emergence of resistance to tigecycline was screened on freshly prepared agar supplemented with 2 mg/L of TIG. For in vivo evaluation of antibiotic activities, chronic localised MRSA infections were established for 2 weeks before therapy, by inoculating in each TC (s.c. implanted in rats) 10^6 CFU of strain MRGR3.
Results: MIC and MBC of VAN for strain MRGR3 were 1 and 2 mg/L, respectively. MIC of TIG for strain MRGR3 was 0.5 mg/L (upper susceptibility breakpoint), albeit not influenced by broth supplementation with 50% TC fluid. Incubation of strain MRGR3 for 24 h with supra-MIC levels (1, 2, and 4 mg/L) of TIG led to a 2–3 log10 decrease in CFU/ml. BID s.c. administration of TIG (7 mg/kg) produced TC fluid TIG levels exceeding its MIC for strain MRGR3 for at least 75% of each dosing interval.
BID s.c. administration of VAN (50 mg/kg) yielded peak and trough, TC fluid levels of 12 and 2 mg/L, respectively. Before therapy, CFU counts of MRSA MRGR3 were equivalent in the different treatment groups, ranging from 6.70 to 6.92 log CFU/ml of TC fluid (n = 84). After 7 days of therapy with TIG or VAN, average counts of MRGR3 decreased significantly ($P < 0.01$) by 0.62 ± 0.17 (n = 29) or 0.76 ± 0.18 (n = 27) log CFU/ml of TC fluid, respectively, compared to untreated rats (increase of 0.18 ± 0.19 log CFU/ml of TC fluid; n = 28). Average reductions in CFU counts of rats treated with TIG or VAN were not significantly different. No colonies with increased TIG MICs were detected in TC fluids from TIG-treated rats by the end of the 7-day therapy.
Conclusion: Despite not being a sensu stricto bactericidal antibiotic, TIG exhibited similar in vivo activity to VAN against MRSA chronic foreign body infections, and did not lead to emergence of subpopulations with increased MIC.

P1907 **Intra- and extracellular activity of dicloxacillin and linezolid against small colony variants of *Staphylococcus aureus* in vivo**

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Objectives: The Small Colony Variants (SCV) phenotype of *S. aureus* has been observed in connection with chronic and recurrent infections, and intracellular persistence of SCVs is suspected. The aim was to compare the virulence of the wild type and the SCV phenotypes and their response to Dicloxacillin (DCX) and Linezolid (LNZ) extra- and intracellularly in vivo
Methods: A clinical menadione auxothrophic SCV isolate (OM 1b) and the corresponding wild type (WT) isolate (OM 1a) from a patient with chronic infection were tested in the mouse peritonitis model. The two phenotypes were clonally identical by PFGE. Both phenotypes were DXC and LNZ susceptible.
In vivo studies: After i.p. inoculation in mice of 2.5×10^7 CFU *S. aureus* (OM 1a or OM1b) in 5% mucin either one dose of DXC (1.5 mg/mouse), LNZ (0.5 mg/mouse) or vehicle was given sc 2 h later.. Four and 24 hr after treatment, mice were euthanised; a peritoneal wash in saline was performed and sampled.
Ex vivo separation assay: The total bacterial count (Total) was quantified before divid-ing the sample into two identical fractions. The extracellular bacterial count (Extra) was determined in the supernatant after centrifugation of one of the sample fractions. In the other sample fraction, extracellular bacteria were killed by adding lysostaphin to a final concentration of 15 mg/ml. The cells were lysed in water after lysostaphin wash out and the intracellular count (Intra) quantified. Groups were compared using the Mann-Whitney test.
Results: As shown in the table, CFUs for untreated Total WT vs SCV were not different after 2 h but significantly lower for SCV after 6 h ($P = 0.0023$) indicating slower growth in vivo. Similar lower CFUs were seen for SCV extra- ($P = 0.03$) and intracellularly ($P < 0.0001$). Both

antibiotics displayed activity both extra- and intracellularly, but the reduction in CFUs were lower for the SCV than for the WT in all compartments. DXC showed 0.5–1 logCFU better killing activity than LNZ overall.

	Δ Log(CFU/ml)$_{\text{from 0 hr}}$ (SD)											
	4 hr						24 hr					
	Wild type			SCV			Wild type			SCV		
	total	extra	intra	total	extra	intra	total	extra	intra	total	extra	intra
Vehicle	0.91	1.45	0.58	0.20	0.24	0.24	ND	ND	ND*	–3.39	–3.39	–3.18
	(0.24)	(0.58)	(0.43)	(0.43)	(0.75)	(0.19)				(0.16)	(0.15)	(0.05)
Dicloxacillin	–0.57	–0.82	–0.08	–0.72	–1.24	–0.43	–1.80	–2.51	–1.98	–4.76	–3.54	–2.85
	(0.16)	(0.14)	(0.15)	(0.15)	(0.13)	(0.18)	(0.20)	(0.51)	(0.13)	(0.00)	(0.00)	(0.66)
Linezolid	0.07	0.01	0.40	–0.48	–0.78	0.14	–1.48	–1.70	–1.43	–2.74	–3.29	–2.20
	(0.14)	(0.06)	(0.14)	(0.11)	(0.14)	(0.17)	(0.21)	(0.49)	(0.24)	(0.42)	(0.28)	(0.95)

*Not detected; mice showed signs of irreversible sickness and were euthanised before 24 hrs of infection.

Conclusion: Both the WT and the SCV variant of *S. aureus* resulted in intracellular in-fection, however, already after 6 h the SCV showed significantly slower growth than the WT in vivo. The lower activity in vivo of the two antibiotics against the SCV was probably a result of the slower growth. Both antibiotics were active extra- as well as intracellularly with DXC displaying somewhat better killing activity in both compart-ments.

P1908 Efficacy of fosfomycin alone or combined with imipenem, linezolid and vancomycin, in vitro and in a murine peritonitis model due to two strains of GISA

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Objectives: To study the efficacy of Fosfomycin (FOM) and its combinations with Imipenem (IMP), Vancomycin (VAN) and Linezolid (LZD) in infections due to GISA strains.
Methods: MICs (mg/l) of the *Staphylococcus aureus* strains were: strain A (FOM 4, IMP 32, VAN 4, LZD 1, CLO 1024) and B (FOM 1024, IMP 64, VAN 8, LZD 2, CLO 1024). In vitro time-killing curves were performed over 24 h to test bactericidal effect of FOM, IMP, VAN and LZD and synergy. In vivo studies: Peritonitis in C57BL/6 mice was induced by intraperitoneal inoculation of 10^8 CFU/ml. Four hours later (0 h), mice were sorted in Control (not treated), FOM (100 mg/kg/5 h), IMP (30 mg/kg/5 h), VAN (60 mg/kg/5 h), LZD (35 mg/kg/5 h) and FOM+IMP, FOM+VAN, FOM+LZD, receiving sc therapy over 25 h. At 0 and 25 h, a blood sample was taken to assess bacteraemia rates and peritoneal wash was performed to determine bacterial counts in peritoneal fluid. Mortality was analysed at 25 h.

Bacterial decrease (D log cfu/ml) and bacteraemia rates (Br) at 25 h

Therapy (25 h)	Strain A			Strain B		
	n	D log cfu/ml	Br (%)	n	D log cfu/ml	Br (%)
Control	17	+0.05	100	19	+0.72	100
FOM	14	–2.78[a]	57[a]	3	–0.44	100
IMP	10	–1.80[a]	100	11	–0.46	81
VAN	14	–2.30[a]	93	16	–1.80[a]	81
LZD	15	–2.31[a]	73[a]	18	–2.27[a]	67[a]
FOM+IMP	14	–3.11[a,b]	50[a]	4	–0.61	100
FOM+VAN	14	–2.82[a]	57[a]	12	–2.33[a,c]	50[a]
FOM+LZD	15	– 2.90[a,b]	43[a]	4	–2.30	78.6

[a]$p < 0.05$ vs control; [b]$p < 0.05$ vs VAN; [c]$p < 0.05$ vs IMP (ANOVA).

Results: In vitro, the combination of FOM (Cmax and 1×MIC) plus IMP (1×MIC) were bactericidal and synergistic for strain A. In the animal model at 25 h, for strain A, all therapies were more effective than controls and FOM+IMP and FOM+LZD were significantly better than VAN ($p < 0.05$). For strain B, all treatment groups except IMP were better than controls and FOM+VAN was significantly better than IMP. Bacterial decrease and bacteraemia rates of the different groups are shown in the table.
Mortality in controls was 90% and 100% with strain A and B, respectively, all therapy groups were better than controls except IMP for strain B ($p < 0.05$).
Conclusions: Combinations of FOM were effective in the treatment of infections caused by the hGISA strain. In the treatment of infection caused by the GISA strain only the combination FOM plus VAN was effective.

P1909 Comparative efficacy of iclaprim against wild-type and thymidine kinase-deficient *Staphylococcus aureus* in a mouse abscess model

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Objectives: Iclaprim (ICL) is a bactericidal diaminopyrimidine antibiotic with an extended spectrum of activity and it is notably very potent against important Gram-positive pathogens, including meticillin resistant *S. aureus*. ICL exerts its antibacterial activity by specifically and selectively inhibiting microbial dihydrofolate reductase (DHFR), a key enzyme in the folate pathway synthesis. As for any DAP, the antimicrobial activity of ICL is known to be antagonised by thymidine (dT). Unlike humans, where dT levels are extremely low, several animal species, e.g., rodents, contain high dT levels which can confound evaluation of DAP activity in vivo. Consequently, successful PK/PD studies have not been reported so far. In previous studies it has been shown that Thymidine Kinase (TK)-Deficient *S. aureus* mutants are a useful tool in the study of ICL both in vitro and in vivo. The aim of this study was to verify the utility of these mutants in a mouse abscess model of infection and, consequently, their potential in determining ICL PK/PD relationship.
Method: The efficacy of ICL against wild-type *S. aureus* ATCC 25923 and its TK-deficient mutant AH 1246 was evaluated in immunocompetent CD-1 mice with subcutaneous abscesses. Abscesses were induced by s.c. injection of bacteria on cytodex beads. Both strains were equally virulent. ICL (2–80 mg/kg) was initially administered s.c. once (two hours post infection) and the bioburden determined at 24 hrs post infection. Linezolid (LZD) was used as control. Subsequently, for a preliminary evaluation of PK/PD relationship, mice were treated with 5 doses of ICL fractionated into 1, 2, 3, or 4 doses over a 24 hrs period.
Result: ICL exhibited no effect against the wild-type strain when tested once up to 80 mg/kg. By contrast, ICL was efficacious against the TK-mutant with 1 and 2 log10 CFU reductions at 14.4 and 30 mg/Kg, respectively. LZD gave 1 and 2 log10 reduction at 15.5 and >40 mg/Kg, respectively against both strains. In the second study, fractionation of the ICL dose was assessed only against the TK-mutant. Analysis of the data, showed AUC/MIC as the primary parameter with R^2 of 0.91.
Conclusion: The study confirms the utility of TK mutants in assessing ICL in vivo efficacy. Preliminary PK/PD correlation appears to indicate AUC/MIC as the primary parameter. Further studies with these mutants are warranted for evaluation of PK/PD in animals.

P1910 Efficacy of telavancin against vancomycin-intermediate *Staphylococcus aureus* in the neutropenic mouse bacteraemia model

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Objectives: Infections caused by Gram-positive organisms displaying intermediate susceptibility to glycopeptides such as vancomycin (VISA) are of significant concern in the medical community. Telavancin (TLV) is a novel lipoglycopeptide antibiotic that operates through a unique, multifunctional mechanism of action to produce potent, rapid bactericidal activity against resistant Gram-positive pathogens, including VISA. The

goal of the present study was to compare the efficacy of TLV with vancomycin (VAN) in a murine model of VISA-induced bacteraemia.

Methods: Immunocompromised mice (female non-Swiss albino, 18–25 g) were inoculated intraperitoneally with VISA HIP5836 (10^7 colony-forming units per mL). Starting 4 hours post-inoculation, mice received two subcutaneous doses (once every 12 hours) of vehicle, TLV or VAN. Mouse pharmacokinetic data were generated and used to select doses of TLV (40 mg/kg) and VAN (110 mg/kg) that equated to clinical exposures. Reductions in bacterial titre (in blood and spleen) at 12, 24 and 48 hours post-treatment were quantified.

Results: Telavancin demonstrated potent antibacterial activity against VISA strain HIP5836 in vitro in terms of MIC and in vivo in terms of reduction in blood and spleen bacterial titres compared with vancomycin (Table).

Table. MICs and blood and spleen bacterial titres

	MIC (mg/L)	Blood (log CFU/mL)*	Spleen (log CFU/g)*
Pre-treatment titre	–	5.5±0.41	8.0±0.24
VAN	4.0	3.6±0.69	7.7±0.49
TLV	0.5	1.0±0.0†	3.8±0.16†

CFU, colony-forming units; MIC, minimum inhibitory concentration.
*Titre at the onset of treatment (in pre-treatment titre row) or at 48 h post-treatment (in TLV and VAN rows).
†$p < 0.05$ vs pre-treatment titre and VAN.

Conclusions: TLV is more efficacious than VAN in a mouse model of VISA bacteraemia infection.

P1911 Efficacy of linezolid vs. vancomycin in the treatment of an experimental meningitis model caused by meticillin-resistant *Staphylococcus aureus*

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Objective: To compare in vivo the activity of linezolid (LZD) and vancomycin (VAN) in a meningitis model in rabbits, caused by meticillin-resistant *S. aureus* (MRSA).

Methods: In vitro: MIC of VAN and LZD was determined for 23 MRSA strains. The bactericidal activity (time-kill curves; 1, 2 and 4×MIC) was studied for 4 strains (MRSA 8, 10, 13 and 17). In vivo: New Zealand rabbits (2.5–3 kg) were used, with an inoculum of 8.5–9 Log10 cfu/mL. PK/PD parameters (blood and CSF) were determined (Cmax [mg/L]; AUC [mg.h/L]; t1/2 [h]; t>MIC [h]; AUC/MIC) after a single dose of 20 mg/kg of each antimicrobial on infected rabbits. Animals were grouped in untreated (CON), LZD (20 mg/kg) every 3 hours (4 doses) and VAN (20 mg/kg) every 12 hours (1 dose). CSF variables analysed at 0, 6 and 12 h of treatment: bacterial concentration (Log10 cfu/mL), leukocytes (cels/mL), lactate (mmol/L), proteins (g/L); qualitative blood cultures were made at 0 and 12 h. Statistical Analysis: Wilcoxon, Mann-Withney, and Chi-square tests.

Results: In vitro: MIC (mg/L) MRSA 8, 10, 13 and 17: LZD=4, VAN=1. Bactericidal activity: MRSA 8: LZD (2×MIC) and VAN (1×MIC, 2×MIC, 4×MIC); MRSA 10: VAN (2×MIC, 4×MIC); MRSA 13: VAN (2×MIC, 4×MIC); MRSA 17: VAN (4×MIC). In vivo: PK/PD (Cmax, AUC; t1/2; t>MIC; AUC/MIC): Blood LZD (20 mg/kg) 12.49, 14.7, 0.67, 1.37; 3.68; Blood VAN (20 mg/kg) 51.2; 120.81, 3.26; >6; 120.81; CSF LZD (20 mg/kg) 7.65, 13.25, 1.03, 1.31, 3.31; CSF VAN (20 mg/kg) 3.2; 14.52; –; >6; 14.52.

Bacterial concentration (0, 6, 12 h): CON (4.85, 4.93, 4.49), LZD (5.05, 4.02, 4.36), VAN (4.85, 4.83, 4.83). Leukocytes: CON (2750, 5610, 3960), LZD (2830, 4230, 2490), VAN (10.24, 22.70, 22.39). Lactate: CON (8.46, 22.48, 22.35), LZD (9.58, 14.48, 12.13), VAN (10.24, 22.70, 22.39). Proteins: CON (0.96, 5.34, 3.95), LZD (1.90, 8.46, 8.65), VAN (2.35, 7.40, 5.12). Sterile blood cultures: CON (90.9%, 81.8%), LZD (90%, 100%), VAN (100%, 100%).

LZD reduced bacterial concentration at 12 h with respect to 0 h ($p < 0.05$). Respect to lactate levels LZD reduced the CSF lactate (vs. control group at 12 h [$p < 0.05$] and vs. VAN group at 6 and 12 h [$p < 0.05$]).

Conclusions: In this model of murine meningitis by MRSA, linezolid was better than vancomycin in reducing the CSF bacterial concentration and lactate levels. It may be an alternative to vancomycin in meningitis caused by MRSA strains.

P1912 Efficacy of levofloxacin alone and in combination with rifampin in staphylococcal experimental foreign-body infection

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Objectives: Since levofloxacin at high doses (LHD) was more active than at conventional doses (LCD) and was the best therapy alone in a rat-model of foreign-body infection (FBI) by *Staphylococcus aureus* (Sa), we tested how these differences affect in the activity of their respective combinations with rifampin (R) in vitro and in vivo.

Methods: In vitro studies. MICs and MBCs (mg/L) of Sa ATCC 29213 were respectively: 0.5, 1 (levofloxacin) and 0.015, 0.12 (R). 24 h kill-curves (KC) were performed in the log- (LP) and the stationary-phase (SP). Animal studies. Two Teflon tissue-cages with 2 cover-slips (CV) each were implanted in rats; after 3 weeks, tissue-cage fluid (TCF) was infected with Sa and, 3 weeks later, therapy was administered for 7 days (7dT) in all cases and prolonged to 14 days (14dT) for LHD alone and with R. Therapeutic groups (mg.kg/h): LHD (100/24; PD values equivalent to human ones for 1000 mg/d), LCD (50/24; PD equivalent to 500 mg/d), LHD+R (25/12), LCD+R and controls (C). Criteria of efficacy: (i) differences in TCF bacterial counts between the beginning and the end of treatment (Δlog UFC/ml); (ii) bacterial counts in CV at the end of 14 dT. The presence of levofloxacin- or R-resistant strains was screened.

Results: KC in LP showed antagonism with all combinations of levofloxacin at concentrations ⩾1 mg/L and R. KC in SP showed indifference with the combination of peak concentrations of LCD (4 mg/L)+R and tend to antagonism with LHD (12 mg/L)+R. The Δlog UFC/ml at the end of 7dT were (n): LHD –2.08 (50), LCD –1.24 (20), LHD+R –1.55 (44), LCD+R –1.51 (20) and C 0.33 (22); all groups were better than C ($p < 0.05$, T-test), LHD was the best therapy ($P < 0.03$ vs LHD+R, $P < 0.01$ vs LCD). At the end of 14dT the Δlog UFC/ml in TCF were (n) LHD –3.84 (21), LHD+R –3.37 (21) and C 0.40 (15), and bacterial counts in CV (logCFU/ml) were 2.24 (LHD), 3.36 (LHD+R) and 5.4 (C); both groups were significantly better than C and LHD better than LHD+R ($P < 0.03$) in CV. No resistant strains were detected in either group at the end of 7dT and 14dT.

Conclusions. LHD confirmed the great efficacy against FBI by Sa, it was the best treatment and no resistant strains appeared. The addition of R to LHD showed antagonism effect; the clinical relevance of these findings should be further evaluated. In contrast, if R is going to be used, the dose of levofloxacin did not contribute significantly to the efficacy of the combination and both doses protect against resistance.

P1913 Aqueous humor concentration of topically administered voriconazole in rabbits

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Objectives: To investigate penetration of voriconazole into the aqueous humor (AH) of rabbits after topical administration.

Methods: Voriconazole 1% solution was applied onto rabbits cornea at different time intervals: single drug application with AH sampling after 30 min, one hour, two hours, 3 hours and 6 hours, respectively. In addition, we evaluated AH samples after continuous topical

application of voriconazole every 30 min after 1, 2, 4 and 6 hours. Furthermore, following continuous drug application every hour we analysed voriconazole concentration after two hours, 3 hours, 4 hours and 6 hours. All samples were analysed by high performance liquid chromatography (HPLC)-UV.
Results: A single application showed a maximum peak in AH of 3.58 μg/ml (N = 9) after 30 min and within three hours the concentration decreased to 0.04 μg/ml (N = 11). Application of voriconazole every half an hour resulted after two hours in a peak value of 6.73 μg/ml (N = 10), decreased after four hours to 6.19 μg/ml (N = 10) and was constant at six hours (6.12 μg/ml, N = 6). With hourly administration no such high values were detected. The maximum value was 2.06 μg/ml (N = 8) and was reached after four hours.
Conclusion: In AH, therapeutic drug levels can be reached that cover the minimum inhibitory concentrations (MIC) of most fungi. To achieve a sustained high level of voriconazole as an effective antifungal therapy for corneal keratitis, voriconazole should be topically administered every 30 min.

P1914 Efficacy of isavuconazole, voriconazole and fluconazole in neutropenic murine models of disseminated *Candida tropicalis* and *Candida krusei*

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Objectives: Isavuconazole (ISA) is the water-soluble prodrug of triazole BAL8557. We examined the dose response of ISA, voriconazole (VCZ) and fluconazole (FLU) on the tissue burden (TB) after 4 and 7 days infection in temporarily (TN) and persistently neutropenic (PN) mice with disseminated *Candida tropicalis* (CT) or *Candida krusei* (CK) infection.
Methods: Male CD1 were immunosuppressed using either 1 dose (TN) or two doses (PN) of 200 mg/kg cyclophosphamide. Mice were infected IV with 6.8 (TN) or 5.2×10^4 (PN) CT, or 1×10^7cfu/mouse CK and treated 5 hours later with solvent, oral ISA (6, 15, 30, 60, 90, 120, 150 mg/kg active compound), IV VCZ (5, 20, 40 mg/kg plus grapefruit gavage BD) or oral FLU (15, 50, 150 mg/kg) BD. 101 hours (CT and CK) or 7 days (CT) post infection mice were killed, kidneys & brains (CK only) removed for culture.
Results: Solvent controls developed a non-lethal infection with high burdens in all models (CT 1.25×10^7–2.1×10^8 in kidneys; CK 4.1×10^5–3.8×10^6 in kidneys and 1.7–4.9×10^6 log10cfu/g in brain.
For CT, TN model: ISA (>6 mg/kg), VCZ (>5 mg/kg) and FLU (all doses) dose dependently reduced TB ($P < 0.05$) after 4 and 7 days. ISA Emax was 60 mg/kg (2.3log10) was similar to FLU Emax (50 mg/kg) but was superior to VCZ (40 mg/kg). ISA >60 mg/kg was superior to all doses VRC. PN model: ISA (all doses) and FLU (all doses) dose dependently and VCZ (40 mg/kg only) reduced TB ($P < 0.05$) in 4 day kidney burden. For 7 day TB ISA (>30 mg/kg), VCZ (all dose >5 mg/kg) and FLU (all doses) dose dependently reduced TB. ISA Emax achieved at >60 mg/kg. No treatment sterilised kidneys.
For CK, TN model: ISA, VCZ and FLU dose dependently reduced kidney burden ($P < 0.05$ all doses). VCZ 40, ISA 120 & 150 were superior to other treatments in reducing kidney burden. ISA (all doses) and VCZ (40 mg/kg) reduced brain burden ($P < 0.05$). FLU (all doses) and VCZ (5 & 20 mg/kg) had no significant effect on brain burden. VCZ 40, ISA 120 & 150 were superior at reducing brain burden but FLU had no significant effect.
PN model: ISA (all doses), VCZ (all doses) and FLU (150 mg/kg) reduced kidney burden ($p < 0.05$) with ISA >90 mg/kg and VRC 40 mg/kg superior. In the brain ISA and VCZ (all doses) reduced tissue burden ($p < 0.05$) FLU had no significant even at 15 mg/kg.
Conclusions: ISA significantly lowered kidney (CT & CK) & brain burden (CK) and was at least as effective as VCZ. ISA was much more effective than FLU at reducing brain burden (CK). ISA had maximal effect by 60 mg/kg and was partially effective at all doses >6 mg/kg.

P1915 Correlation of in vitro MIC and MFC against isavuconazole, voriconazole and amphotericin B of *Aspergillus* with in vivo outcome in mice with disseminated aspergillosis

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Background: Assessment of susceptibility of *Aspergillus* isolates is commonly performed using the CLSI M38A protocol. In this protocol breakpoints are assigned using the MIC whilst in many cases there are large differences between the MIC and MFC. In this study we examined strains with large differences between the MIC and MFC using time-kill experiments and murine models of disseminated aspergillosis to determine which parameter better reflects the response to antifungal therapy.
Methods: MICs and MFCs (99.9%) were determined according to the CLSI M38A guidelines and a range of strains in which MIC were similar to MFC and also MIC ≫ MFC were selected. In vitro time-kill curves using RPMI 1640 + 2% glucose were set up using 9 strains of *Aspergillus* (table 1) exposed to isavuconazole (ISA), voriconazole (VCZ) and amphotericin B (AmB) and followed for 48 h. Four strains of *A. fumigatus* (AF) one *flavus* (AFL) and one *terreus* (AT) were used to study the in vivo effect. Male CD1 mice 22–24g were immunosuppressed with a single dose of 200 mg/kg cyclophosphamide IV then 3 days later infected IV with a sub-lethal inoculum of *Aspergillus* (AF4, A1163, AF210, AF293, AT49, AFL8). Both ungerminated and partially germinated conidia were used for AF293. 4 hours after infection mice were treated once daily with either vehicle, ISA (oral), VCZ (IV) plus grapefruit juice (oral) AmB (IP), or caspofungin (CAS) (IV) for 3–4 days. 72–96 h post infection mice were euthanised and the kidneys removed for quantitative culture.
Results: MICs and MFCs are shown in table 1. Four strains of AF with low MIC and MFCs (AF4, AF82, A1163 and AF210) were rapidly killed in vitro. Other strains with low MIC but high MFCs demonstrated much slower clearance using in vitro time-kills (for ISA $p = 0.046$ for MIC ≥ 4 mg/L AmB and VCZ NS). In vivo strains generated moderately severe non-lethal infections with heavy and reproducible tissue burdens in the kidneys. The strains with low MIC and MFCs (A1163 and AF210) had very low residual tissue burdens following treatment with all antifungals. In contrast strains with high MFCs had terminal tissue burdens very similar to control animals.

Table 1

Strains	Isavuconazole				Voriconazole				Amphotericin B			
	MIC	MFC	Time kill	In vivo	MIC	MFC	Time kill	In vivo	MIC	MFC	Time kill	In vivo
AF19	0.5	8	Intermediate		0.25	2	Intermediate		0.25	8	Slow	
AF1108	0.5	16	Slow		0.25	2	Intermediate		0.5	8	Slow	
AF4	0.25	0.5	Rapid	Cleared	0.125	1	Intermediate		0.5	2	Rapid	
AF82	0.25	2	Intermediate		0.25	1	Rapid		0.25	1	Rapid	
Al163	0.25	0.5	Rapid	Cleared	0.25	2	Rapid	Cleared	0.25	2	Rapid	Cleared
AF210	0.5	8	Rapid	Cleared	0.25	2	Rapid	Cleared	0.25	2	Rapid	Cleared
AF293	0.5	16	Slow	Not cleared	0.25	4	Slow	Not cleared	0.25	8	Slow	Not cleared
AT49	0.25	8	Slow	Not cleared	0.25	8	Slow	Not cleared	1	2	Slow	Not cleared
AFL8	1	4	Slow	Not cleared	0.25	2	Slow	Not cleared	0.5	1	Slow	Not cleared

Conclusion: In this study all strains had similar MICs but demonstrated a wide range of MFCs. The MFC better correlated with the rate of kill in vitro. Additionally the high MFCs of two strains (AF293 and AT49) were generally reflected by higher terminal tissue burden in murine models of disseminated aspergillosis.

P1916 Efficacy of liposomal amphotericin B, amphotericin B deoxycholate or caspofungin in the treatment of experimental *Candida glabrata* catheter infection using the antifungal-lock technique

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Objective: *Candida glabrata* (CG) is the third most frequent species of *Candida* causing bloodstream infections; many cases are related to catheter infections (CI). The ability of *Candida* to form biofilms difficult the treatment of infected catheters. Catheter removal is sometimes difficult despite it is recommended to manage CI due to *Candida*. The aim of this study was to evaluate the efficacy of liposomal amphotericin B (LAB), caspofungin (CAS) and amphotericin B deoxycholate (DAB) in the treatment of *C. glabrata* CI using the antifungal-lock technique (ALT).
Methods: New Zealand rabbits were surgically implanted with a silicone central venous catheter trough the jugular vein. After insertion, the catheter was filled with 0.35 mL of broth culture (antibiotic medium 3 + 8% glucose) containing a 10^7 cfu/mL suspension of *C. glabrata* (strain CG 171) isolated from a human case of catheter-related bloodstream infection. 48 h later the inoculum was removed and catheters were filled with the appropriate antifungal solution for 48 h. Animals were randomised into the following groups: Untreated Control; LAB 5 mg/mL; CAS 5 mg/mL; and DAB 5 mg/mL. At the end of the treatment period the animals were sacrificed and CI was assessed using transcatheter blood culture, roll-plate, flushing and sonication techniques. Differences in rate of positive catheters and log cfu were analysed using Chi-square and Mann-Whitney tests as appropriate. Samples showing no growth were assumed to be log=0.3. $P < 0.05$ was considered significant.
Results: Results are reflected in table 1.
Conclusions: All ALT treatments reduced significantly the total number of culture positive catheters as well as the log ufc compared to untreated controls. No significant differences were found between the three antifungals. Nevertheless, the number of culture positive treated catheters remains between 30 and 60%underlinig the need for further studies on conservative strategies for the treatment of experimental *C. glabrata* catheter-related infections.

Table 1

Group	N	N+ (% of total)	flushing, mean log ufc (95% CI)	sonication, mean log ufc (95% CI)
Control	13	13 (100)	4.38 (3.45–5.31)	4.83 (4.01–5.64)
LAB 5 mg/mL	13	6 (46.2)*	0.77 (0.22–1.33)*	0.82 (0.37–1.28)*
CAS 5 mg/mL	9	3 (33.3)*	1.04 (0.03–1.62)*	1.41 (0.09–2.74)*
DAB 5 mg/mL	14	8 (57.1)*	1.46 (0.38–2.55)*	1.26 (0.42–2.10)*

N: total number of animals; N+: total number of culture positive catheters; *$p < 0.05$ vs control.

P1917 Mecillinam is active against ESBL-CTX-M producing *E. coli* in vivo in experimental UTI

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Objectives: The prevalence of ESBL-producing *E. coli*, especially the CTX-M types, are increasing in the Northern Europe, mostly occurring as causes of urinary tract infection (UTI). Mecillinam (Mec) is active against many ESBL-producing *E. coli* in vitro (Livermore et al. JAC, 2006), so we wanted to investigate the effect in vivo – in comparison with meropenem (Mer) and ciprofloxacin (Cip) – in an experimental UTI model in mice.
Materials and Methods: We used two clinical UTI strains of *E. coli*, one producing CTX-M9 (Vec23), and one producing CTX-M15 (DSA 443). The MIC against Mec, Mer and Cip was determined by Etest og microtitre broth dilution. Presence of type-1 fimbriae was assayed by the yeast-agglutination assay. HsdWin:CFW1 female mice were treated for 3 days with 5% glucose in their drinking water. At day 0, they were anaesthesised with Zoletil/Torbugesic and 50 mcl of a 10E9 cfu/ml solution in saline inoculated into the bladder via urethral catheterisation with a 0.63 mm tube. Treatment with doses resulting in human-like PK (Mec, 0.5, Mer, 1, and Cip, 0.18 mg/mouse vs. saline treated controls) was initiated day 1 and continued BID for 3 days. At day 4, all mice were killed for sampling of urine, bladder and kidneys for CFU determination.
Results: MIC's (mg/l) for Mec, Mer and Cip were for Vec23: 2, 0.5 and >32, and for DSA 443: 0.5, 0.32 and 0.125, respectively. Mecillinam and meropenem were equally active against both isolates, and both better than ciprofloxacin, after 3 days of treatment: *E. coli* Vec23: (median CFU/ml or CFU/kidney); *, $P < 0.05$ as compared to control), Urine: Control: 3.4×10^3, Mec: 0*, Mer 0*, Cip: 10^6. Kidneys: Control: 1.6×10^2, Mec: 0*, Mer: 0*, Cip: 1.1×10^3. *E. coli* DSA 443: Urine: Control: 1.03×10^5, Mec: 2.0×10^3*, Mer: 0*, Cip: 85. Kidneys: Control: 6.5×10^2, Mec:0*, Mer: 5.5*, Cip: 15.5*. In total (both *E. coli* strains) Mec sterilised urine in 7/12 mice, Mer 10/12, Cip 0/12; Mec sterilised kidneys in 9/12 mice, Mer 8/12, and Cip 4/12. There was no or little effect on bladder CFUs, as shown for all other antibiotics.
Conclusion: The Vec23 strain was R for Cip explaining the missing effect of this drug. Mecillinam was as effective as meropenem both regarding reduction of CFUs in urine and in kidneys. Both drugs sterilised around 60–80% of mice urine and kidneys of the ESBL-producing *E. coli*. Mecillinam is a promising drug for treatment of UTI caused by ESBL-producing *E. coli*, and clinical trials are warranted.

P1918 Are carbapenems effective against carbapenem-susceptible, metallo-β-lactamase producing Enterobacteriaceae? Results from an animal model

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Background: Although metallo-β-lactamases (MBLs) hydrolyse most β-lactams including carbapenems, MBL-producing Enterobacteriaceae very often remain susceptible to carbapenems in vitro. The aim of this study was to assess the in vivo efficacy of imipenem (IMP), meropenem (MEM), ertapenem (ERT) and aztreonam (AZT) against a carbapenem-susceptible VIM-1-producing clinical *E. coli* strain in a rabbit intraabdominal abscess model.
Methods: MBL-production in *E. coli* 6641 was confirmed with PCR and sequencing. MICs were determined with microdilution method. Rabbits were inoculated intraperitoneally with 10^8 CFU/ml of *E. coli* 6641 and were assigned to receive no treatment (controls) or i.v. IMP-cilastatin 70 or MEM 125 or ERT 60 or AZT 70 mg/Kg/12 h. Antibiotic serum levels were determined using a microbiological assay. A total of 8 doses were administered before sacrifice. The abscesses were harvested and quantitatively cultured. Mean viable bacterial counts of each treatment group were compared using Kruskal-Walis test.
Results: *E. coli* 6641 carried blaVIM-1 and had IMP, MEM, ERT and AZT MICs of 1, $\leqslant$0.25, 1.5 and $\leqslant$0.25 μg/ml, respectively. Serum levels confirmed that T>MIC was achieved for $\geqslant$50% of dosing interval for all tested antibiotics. The log10 CFU/g (mean +-SD) of pus were as follows: controls (16) 8.71+-1.34 ($p < 0.001$, vs all other groups), IMP (15) 5.30+-2.73, MEM (15) 4.24+-2.44, ERT (16) 3.17+-1.85 ($p = 0.022$, vs IMP), AZT (15) 3.62+-3.05. Mortality among AZT-treated rabbits (16.7%) was significantly reduced compared to controls (69.2%) ($p = 0.006$). Four animals in the AZT group (26.7%) had culture-negative pus.
Conclusion: In the rabbit experimental model, carbapenems were proved equally effective with AZT in the treatment of intraabdominal infection due to a carbapenem-susceptible VIM-1 producing clinical *E. coli* strain.

P1919 Comparison of quinupristin-dalfopristin and vancomycin in the treatment of experimental endocarditis due to ampicillin- and gentamicin-resistant *E. faecium*

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Objectives: Drug resistance among enterococci necessitates the search for new treatment options.
In this study we tested the efficacy of vancomycin and quinupristin-dalfopristin in a rabbit model of experimental endocarditis due to an ampicillin (AMP) & gentamicin (GEN) resistant and glycopeptides susceptible clinical isolate of *Enterococcus faecium*.
Methods: We used the left side experimental endocarditis model. We inoculated animals with *E. faecium* one day after the insertion and fixation of a polyethylene catheter into the left ventricle. Thirty-six hours later they were randomly assigned to a control group (C), and groups receiving quinupristin-dalfopristin 30 mg/kg/q8 h IM x 5 days (QD) or vancomycin 50 mg/kg/q12 h IM x 5 days (V). All animals were sacrificed at the end of treatment. Therapy response was determined by blood cultures and quantitative (log10 cfu/gr of tissue) cultures of aortic valve vegetations, liver, spleen, kidney and brain.
Results: The results obtained of the different therapeutics groups are expressed in the Table.

Groups	Vegetation[a,b]	Liver[a,b]	Kidney[a,b]	Spleen[a,b]	Brain[a,b]
C	7.86±0.91 (0/10)	5±2.42 (0/10)	5.8±2.54 (0/9)	5.23±2.67 (0/9)	4.63±1.85 (0/9)
QD	7.41±1.11 (0/8)	5.15±1.05 (0/8)	4.96±1.4 (1/8)	5.11±1.42 (1/8)	4.6±1.19 (1/8)
V	6.19±0.66 (0/10)	4.07±0.79 (0/10)	3.85±0.89 (1/10)	3.8±0.94 (1/10)	3.77±0.57 (1/10)
Mann-Whitney 2-tailed test	C vs QD p = 0.015 C vs V p <0.0001 V vs QD p = 0.043	C vs V p = 0.023 C vs QD p = ns V vs QD p = ns	C vs V p = 0.01 C vs QD p = ns V vs QD p = ns	C vs V p = 0.01 V vs QD p = 0.043 C vs QD p = ns	V vs QD p = 0.043 C vs V p = ns C vs QD p = ns

[a] Mean±SD $\log_{10}$ CFU/g of tissue.
[b] No. of sterile vegetations/No. of treated animals.
All the blood cultures of the QD and V group at the end of treatment were negative.

Conclusions: Although both regiments reduce significantly the bacterial count per gram of vegetations, treatment with V was found to be more potent than QD. Also animals treated with V had lower bacterial counts per gram of liver, brain and spleen tissue.

P1920 *Pseudomonas aeruginosa* chronic lung infection model; therapeutic efficacy of ciprofloxacin-betaine after two treatment cycles

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Objectives: Patients with cystic fibrosis (CF) are at particular risk of *Pseudomonas aeruginosa* (PA) infections. In patients with CF, progressive obstructive lung disease due to chronic infection with PA is the primary cause of morbidity and mortality. Maintenance therapy with inhaled antibiotics reduces pulmonary exacerbations and hospitalisations. A dry powder formulation of ciprofloxacin (CIP)-betaine is currently being developed for maintenance therapy to combat chronic PA infections in patients with CF. This study reports the development of a rat model of PA chronic lung infection and the efficacy of CIP-betaine in the model.
Methods: A rat model of airway infection, using agar beads containing PA (prepared according to standard methodology) was used. On days 1 and 8 of the study, anaesthetised rats were given intratracheal (IT) instillation of 0.1 mL PA in 2% agar beads. A concentration of 2×10^7 colony forming units (CFU) were instilled per rat at each infection cycle. Once-daily IT-treatment with CIP-betaine 2.5 mg/kg was performed on days 5, 6 and 7, with a second treatment cycle on days 13, 14 and 15. On days 5, 8, 13 and 16, lungs were removed aseptically and homogenised (n = 5 animals per treatment per sampling point). The viable bacterial counts (measured as CFU) were determined by plating serial 10-fold dilutions on agar plates.
Results: Rats infected with PA in agar beads on days 0 and 8 showed constant levels of infection up to day 16 post-instillation (approximately 10^5 CFU/mL determined in lung homogenate). Two treatment cycles with CIP-betaine achieved a significant reduction in CFU in the lungs compared with an untreated infection control group. The reduction in CFUs was more pronounced after the second treatment (mean reduction in log CFU of 1.9, with eradication achieved in four of five animals) compared with the first treatment (mean reduction in log CFU of 1.7, with eradication achieved in two of five animals).
Conclusions: A PA chronic lung infection model was established by use of the agar beads methodology, in which lung infection could be maintained for up to 16 days. CIP-betaine administered over two treatment cycles significantly reduced the number of PA cells in the lungs.

P1921 Daptomycin is superior to penicillin for treatment of murine soft tissue infections caused by group A streptococci (*Streptococcus pyogenes*)

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Objectives: Penicillin (PEN) is the time-honoured therapy for infections caused by *Streptococcus pyogenes* (group A streptococcus, GAS). However, PEN alone is not considered adequate therapy for severe GAS soft tissue infections because of the “Eagle Effect” (failure of PEN but not clindamycin (CLI) in severe soft tissue infection in animal models). The mechanisms responsible for the Eagle Effect are poorly understood but may include the failure of PEN to kill stationary phase bacteria, inhibit toxin production, or blunt the host response to GAS. It is not known whether antibiotics other than clindamycin are also superior to β-lactams for treatment of severe GAS soft tissue infections. Daptomycin (DAP) is a novel cyclic lipopeptide antibiotic active against Gram-positive organisms that is bactericidal but does not appear to trigger rapid bacterial lysis. We hypothesised that DAP would be superior to PEN for treatment of severe GAS soft tissue infections in mice.
Methods: We injected groups of 12 SKH1-Charles River hairless (immunocompetent) mice in the right upper thigh muscle with 1×10^8–1.5×10^9 cfu of an M3T1 clinical strain of GAS. We initiated antibiotic treatment (either DAP or PEN) at either 18 hours or 40 hours after bacterial inoculation with 1.5×10^9 and 10^8 cfu respectively. All animals were observed daily, and lesion size and character were monitored.
Results: Mice injected with either 10^8 or 1.5×10^9 GAS consistently developed soft tissue infections; lesions were more extensive and more necrotic in mice given the higher inoculum. Lesion volume in DAP-treated animals was consistently less than that in the PEN-treated animals: 36% less in the animals inoculated with 10^8 organisms and 64% in the mice inoculated with 1.5×10^9 organisms. Lesions in the DAP treated animals were also much less necrotic in appearance.
Conclusions: We found that mice infected with an M3T1 strain of GAS and treated with DAP developed smaller, less severe soft tissue lesions compared to mice treated with PEN. We conclude that daptomycin deserves additional study as potential therapy for severe GAS soft tissue infections.

P1922 Impact of the interaction of R207910 with rifampicin on the treatment of murine tuberculosis

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Objectives: New drugs are needed to shorten the duration of TB treatment. R207910, a diarylquinoline, is very active against *Mycobacterium tuberculosis* both in vitro and in mice. In healthy volunteers, co-administration of rifampicin induces increased metabolism of R207910 and as a result, exposure to R207910 is decreased by 50%. A similar drug-drug interaction is not observed in mice, and the efficacy in mice may therefore overestimate the efficacy in patients. The aim of this study is to evaluate the impact of decreasing doses of R207910 when used in combination with isoniazid (H), rifampicin (R) and pyrazinamide (Z) on the efficacy in the mouse model.

Methods: Mice were infected intravenously with 10^7 *M. tuberculosis* and were treated 2 weeks later with the combination of HRZ and R207910 given at 3, 6.25, 12.5 or 25 mg/kg for 2, 4, 6 and 8 weeks. The CFU counts in the spleens were measured and the proportion of mice having negative cultures were determined.
Results: When RHZ was combined with 25 mg/kg of J, all mice had culture negative spleens after 6 weeks of treatment. When RHZ was combined with 12.5 mg/kg of J, almost all mice had culture negative spleens after 8 weeks of treatment. When RHZ was combined with 6 mg/kg of J, 4 of 6 mice had culture negative spleens after 8 weeks of treatment R207910 given at 3 mg/kg did not improve the treatment of mice when combined with RHZ.
Conclusion: In mice, the dose of R207910 could be decreased from 25 to 12.5 mg/kg without loosing too much bactericidal efficacy. Neither the use of a strong background regimen (RHZ) nor the presence of rifampicin is expected to conceal the efficacy of R207910 in patients.

P1923 Effect of vancomycin versus rifampicin in combination with ceftriaxone on inflammation and hearing loss in experimental pneumococcal meningitis

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Background: Up to 30% of survivors of bacterial meningitis (BM) suffer from persisting hearing loss (>90dB) due to damage to the inner ear. Vancomycin has become an important antibacterial treatment option for BM due to its efficacy against *Streptococcus pneumoniae* strains that are resistant to other antibiotics. However recent studies reported increased ototoxic effects of vancomycin combined with ceftriaxone in BM.
Aims: In the presented study we assessed the effect of combination therapies of ceftriaxone with either vancomycin or rifampicin on cytokine levels in the cerebrospinal fluid (CSF) 6 h after initiation of antibiotic therapy and at 21 days after infection we assessed the incidence and extent of hearing loss by auditory brain stem response (ABR) and neuronal density in the spiral ganglion of the inner ear.
Methods: Eleven days old Wistar rats (n = 46) were injected intracisternally with 10μl saline containing 2×10^6 cfu/ml *S. pneumoniae* (serogroup 3) or sterile saline as controls (n = 10). CSF was obtained by puncture of the cisterna magna to document infection at 18 h after infection and to assess cytokine levels at 24 h after infection. Antibiotic treatment was initiated 18 h after infection. Rats were randomised for 10 days treatment with either vancomycin (60 mg/kg i.p.; bid) in combination with ceftriaxone (100 mg/ml s.c.; bid) or for treatment with rifampicin (20 mg/kg i.p.; bid) in combination with ceftriaxone. Twenty days after infection ABR was measured, animals were sacrificed and both cochleae processed for histomorphometry.
Results: All infected animals showed increased CSF concentrations of IFN-gamma, TNF-alpha, IL-1alpha, IL-1beta, IL-6 and IL-10. Of all infected animals 94% demonstrated a significant hearing loss ($P < 0.0001$, compared to mock infection). Neuronal density between 1882 and 1099 cells /mm2 was associated with normal hearing capacity. Hearing started to decline at a neuronal density of 1052 cells/mm2 and below 746 cells /mm2 rats were functionally deaf. Combination of ceftriaxone with vancomycin vs. ceftriaxone with rifampicin did not result in significant differences between the two treatment groups for all parameters evaluated.
Conclusions: The combination of vancomycin plus ceftriaxone versus rifampicin plus ceftriaxone for therapy of experimental pneumococcal meningitis did not produce significant differences in CSF inflammation and the incidence and severity of hearing loss.

P1924 Efficacy of tigecycline versus colistin in a mouse model of pneumonia caused by carbapenem-resistant *Acinetobacter baumannii*

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Objectives: To compare efficacy of tigecycline versus colistin as monotherapy or combination therapy with rifampin in a mouse model of pneumonia caused by carbepenem-resistant *A. baumannii*.
Methods: C57BL/6 mice rendered transiently neutropenic were infected intratracheally with 10^6 CFU of carbapenem-resistant *A. baumannii*. Mice were treated with tigecycline, colistin, rifampin, tigecycline plus rifampin, or colistin plus rifampin. The survival rates of mice, the rates of bacteraemia eradication, and lung colony counts (logCFU/g (mean±standard deviation)) were compared between each group at 24 h and 48 h after inoculation.
Results: Tigecylcine plus rifampin were synergic and the most active regimen among time-kill curves of 0.5 and 1 MIC for 24 h incubation. The untreated control group showed 73% and 36% of cumulative survival rates and 100% of bacteraemia rates at 24 h and 48 h. The survival rates of mice treated with antimicrobial agents except colistin monotherapy were significantly higher than that of the control group ($p < 0.05$). Tigecycline monotherapy showed significantly higher rates (60%) of the bacteraemia eradication at 48 h than the colistin monotherapy that were unable to eradicate the bacteraemia at all ($p < 0.05$). The rates (100%) of the bacteraemia eradication in tigecycline plus rifampin group were the highest among effective antimicrobial treatment groups at 24 and 48 h, but not statistically significant. Tigecycline (10.77±0.28, 9.13±0.93) and colistin (10.42±0.24, 10.60±0.57) monotherapy did not show the effect to reduce the lung colony counts compared to the control group (10.31±0.63, 9.68±0.66) at 24 and 48 h. Rifampin monotherapy showed significantly lower lung colony counts (7.58±0.88, 4.83±0.8) at 24 and 48 h than control group ($p < 0.05$). The lung colony count of two rifampin based combination regimens were signifcantly lower than that of the tigecycline and colistin monotherapy ($p < 0.05$), but not synergic. They were not different between each other.
Conclusion: Rifampin based combination regimens with tigecycline or colistin were effective and comparable to treat mouse model of bacteraemic pneumonia caused by carpabenem-resistant *A. baumannii*.

P1925 Immuno-biochemical comparison of susceptible BALB/c and resistant C57BL/6 mice responses to *Leishmania major* infection

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Objectives: This study has been carried out to compare the pathophysiological signs, production of nitric oxide, serum concentration of microelements and liver enzymes including Aspartate Transaminase (AST), Alanin Transaminase (ALT) and Alkaline Phosphatase (ALP) in two different susceptible BALB/c and resistant C57BL/6 mice infected with *Leishmania major* MRHO/IR/75/ER; as a prevalent strain of cutaneous leishmaniasis in Iran.
Methods: In order to carry out this investigation, mice were assigned into 4 groups of 5 mice as control BALB/c, infected BALB/c, control C57BL/6 and infected C57BL/6. Experimental Leishmaniasis was initiated by subcutaneous injection of promastigotes into the basal tail of test groups. The development of lesions was determined weekly by measuring the two diameters. After 10 weeks, all mice were killed humanly by terminal anaesthesia and target tissues including lymph nodes, spleen and liver from each mouse were removed, weighted and their impression smears were also prepared. Griess microassay method applied for measurement of NO concentration in plasma, liver and spleen suspensions. Serum microelements including Zn and Cu were determined by direct aspiration of 1:10 dilution of serum in deionised water into the Atomic Absorption Spectrophotometer. Serum AST, ALT and ALP were determined by Auto Analyzer Technical RA1000.

Results: NO concentration in plasma, NO2 concentration in tissues, progress of lesion size, proliferation of amastigotes inside macrophages, pathogical signs and biochemical factors in two susceptible and resistant hosts are varied and these variations are depended on mice strain.
Conclusion: Analysis of data from this study revealed an association between RNI levels with the evolution of disease, which had effects on pathological sign and able to modify clinical signs. Zn deficiency in plasma of susceptible BALB/c mice infected with *L. major* indicates possible therapeutic administration of Zn in this form of leishmaniasis. Increase in Cu level in C57BL/6 mice might augment resistant to infection with intracellular pathogens. Our data indicate that Cu/Zn imbalance can be useful marker for dysfunction in leishmaniasis. Alteration of liver enzymes concentration is a consequence of leishmaniasis among BALB/c and C57BL/6 mice. Finally we concluded that differences between susceptible BALB/c and resistant C57BL/6 mice were correlated with genetically induced immuno-biochemical factors.

P1926 Engineered *Bifidobacterium breve* with improved clinical efficacy

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Objectives: To engineer the probiotic strain *Bifidobacterium breve* with improved tolerance to stresses encountered during gastrointestinal transit and improved clinical efficacy.
Methods and Results: Cloning of the listerial derived stress survival genes betL (encoding BetL a compatible solute uptake system linked to improved osmotolerance) and bilE (encoding BilE a bile exclusion system linked to bile tolerance) into the probiotic strain *Bifidobacterium breve*, using the expression vector pNZ8048, significantly improved the strain's ability to cope with a variety of stressful conditions, both external to and within the host.
Heterologous expression of BetL resulted in improved resistance to low and high temperatures and desiccation – ex vivo stresses encountered by probiotics during product (food and/or tablet) formulation and subsequent storage. While expression of BilE gave rise to improved in vitro bile tolerance and resulted in a significant improvement in gastric transit and gastrointestinal persistence in a mouse model. Furthermore, real-time tracking of a luminescent labelled pathogen (*Listeria monocytogenes*) in live animal models of infection revealed a significant reduction in the level of infection when mice were fed *B. breve* expressing bilE compared to the bilE– control strain (Fig. 1) prior to oral inoculation with the pathogen.
Conclusion: It is possible to selectively improve probiotic stress survival, both inside and outside of the host, by heterologous expression of stress survival genes from more physiologically robust strains.
Such genetically engineered 'pharmabiotics' exhibit significantly improved clinical efficacy and may ultimately provide an alternative treatment for recalcitrant, antibiotic resistant infections such as *Clostridium difficile*.

Figure 1. The Xenogen IVIS Imaging System allows in vivo real-time tracking of labelled pathogen and probiotic. Three days post oral inoculation the levels of *L. monocytogenes* reaching the liver were 99.9% lower in animals fed with *B. breve* expressing *betL* (blue) as opposed to the *betL* minus control strain (red). Thus, proving that probiotic clinical efficacy can be dramatically improved by genetic manipulation.

P1927 Determination of the impact of pneumoperitoneum on sepsis severity in an animal model of peritonitis through C-reactive protein and procalcitonin serum levels assessment

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Objectives: C-reactive protein (CRP) and procalcitonin (PCT) consist two substantial inflammator markers. The aim of this study was to evaluate the effect of CO2 pneumoperitoneum, induced for different exposure times, on sepsis cascade through determination of CRP and PCT serum levels.
Methods: In 30 New Zealand rabbits, peritonitis was induced by the cecumligation and puncture (CLP)model. After 12 hours, animals were randomised in 7 groups: group 1; control group, groups 2, 3, 4 and 5; pneumoperitoneum of 10–15 mmHg for 60–180 minutes, groups 6 and 7; laparotomy for 60 and 180 minutes, respectively. Blood samples were collected before CLP, 12 later and 1, 3 and 6 hours after pneumoperitoneum desufflation or abdominal trauma closure to evaluate bacteraemia, C-reactive protein (CRP) and procalcitonin (PCT) levels. Furthermore, 2-day mortality was recorded in all animals. For the assessment of CRP and PCT, 2 ml of blood were collected in pyrogen-free tubes (Vacutainer, Becton Dickinson, Cockeysville, MD) and centrifuged. The serum was kept refrigerated at –70ºC until assayed. Serum levels of CRP were quantitatively determined using an automated immunoturbidimetric assay (Hitachi 912, ROCHE, Basel, Switzerland), with the detection limit of the assay being 0.5 mg/dl.PCT was measured using a commercial immunoluminometric assay (B.R.A.H.M.S. Diagnostics GmbH, Berlin, Germany)which uses two antibodies that bind to two sites (calcitonin and katakalcin)of the procalcitonin molecule, thus ruling out cross-reactivity. The detection limit of the assay is 0.5 ng/ml.
Results: Bacteraemia was induced in all groups. Moreover, in all groups significant increases of CRP and PCT levels were measured at 12 hours following peritonitis induction, with respect to baseline values ($p < 0.05$). Subsequent measurements showed even higher levels of CRP and PCT in all groups. No differences were recorded among groups in association to CRP levels at all time intervals. However, serum PCT reached statistically higher levels ($p < 0.05$) in groups with laparotomy compared to groups with pneumoperitoneum and control group at 6 hours. Survival was lower in groups with laparotomy than in groups with pneumoperitoneum and control group ($p < 0.05$).
Conclusion: In the presence of peritonitis, CO2 pneumoperitoneum applied in usual in clinical practi-ce pressures, even for extended time intervals, attenuates the severity of sepsis and increases survival.

P1928 The Dot/Icm system is essential in the outcome of *Legionella pneumophila* pneumonia in vivo

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Objective: *Legionella pneumophila* (L. p) is a facultative intracellular Gram-negative pathogen responsible for life-threatening pneumonia. The *Legionella pneumophila* Dot/Icm system is an important macromolecular transporter associated with virulence. The aim of the study was to determine the in vivo involvement of Dot/Icm system in L. p pathogenesis for 3 clinical isolates responsible for endemic (L. p Paris) and epidemic (L. p Lens and Philadelphia) cases.
Methods: A/J mice were infected by an intra-tracheal instillation of 106 bacteria using clinical isolates, L. p Paris, Lens and Philadelphia strains (serogroup 1) and their isogenic mutant invalidated in dotB gene. Acute lung injury was assessed in a control group and at 24 and 48 h post-infection by measuring: (i) the protein levels in bronchoalveolar lavage fluids (BALF); (ii) the lung endothelial permeability to radio labelled I125 albumin (Perm-I125); (iii) the alveolar inflammatory response through levels of tumour necrosis factor (TNF)-alpha, interferon (IFN)-gamma, interleukin (IL)-6, IL-12p70 cytokines in BALF. Systemic spread was assessed by blood, liver, kidneys, spleen and brain cultures.

Results: Protein levels in BALF and Perm-I125 were significantly lower at 48 h in the mutant strain infected groups than in the wild type infected groups ($P<0.001$ and $P<0.05$ respectively). Levels of IFN-gamma, IL-6, IL-12p70 in BALF, except TNF-alpha, were significantly lower at 48 h in the mutant strain infected groups than in the wild type infected groups ($P<0.001$). Bacterial localisation in blood, liver, kidneys, spleen and brain cultures were significantly reduced at 24 and 48 h in the mutant strain infected groups than in the wild type infected groups ($P<0.001$).
Conclusion: The Dot/Icm system of endemic and epidemic strains of L. p serogroup1 is a critical virulence factor in vivo, associated with the development of alveolar-capillary barrier injury, alveolar proinflammatory response and bacterial dissemination emphasising its relevance as a major potential therapeutic target.

P1929 Murine model of lung infection by *Parachlamydia acanthamoebae*

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Background: Serological and molecular-based studies support the role of *Parachlamydia acanthamoebae* as a potential new agent of pneumonia. Moreover this intracellular bacteria was shown to resist degradation in human macrophages (i.e the first line of innate immune defence against invading pathogens). To test the third Koch postulate, we investigated whether *P. acanthamoebae* may cause pneumonia in mice.
Methods: Living or heat-inactivated *P. acanthamoebae* bacteria were injected intratracheally to mice. PBS was injected in additional mice (negative control). Mice were sacrificed at days 0, 2, 4, 7, 10, and 21. Non-infected mice sharing the cage of infected mice were sacrificed at days 10, 21 and 46. Histology, immunohistochemistry, qPCR and amoebal co-culture (a cell culture system which uses amoebae as cell background) were performed on lungs. Serological titers were determined by immunofluorescence on sera collected at time of sacrifice.
Results: All mice challenged with living *Parachlamydia* presented signs of pneumonia (purulent $n=11$; interstitial $n=3$) at histology on day 2, 4 and 7. No control mice presented signs of pneumonia. Presence of *Parachlamydia* was confirmed by immunohistochemistry using specific rabbit anti-*Parachlamydia* antibodies. Many *Parachlamydia* were present on day 2, 4 and 7 in lungs of infected mice, which presented interstitial or purulent pneumonia. No control mice (those injected with heat-killed bacteria and those injected with PBS) presented signs of pneumonia.
About 106 parachlamydial genomic DNA copies were detected by qPCR in the lungs of mice injected with living *Parachlamydia*. Mice injected with PBS were negative.
Using amoebal co-culture, we were able to re-isolate living *Parachlamydia* from infected lungs. Control mice were negative.
Serology was negative at day 0, 2, 4 and 7. Anti-Waddlia antibody titers increased to 1/128 at day 14 and 1/512 at day 21 post-infection.
Conclusion: In this study, we established an animal model of lung infection by *P. acanthamoebae*. This confirms the third Koch postulate and strongly supports the role of *P. acanthamoebae* as an agent of pneumonia.

P1930 Experimental models of upper urinary tract infections caused by sexually transmitted diseases isolated or in conjunction with non-specific agents

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Objectives: Experimental models of low urinary tract infections caused by non-specific agents are known. There are no reports which show the role of sexually transmitted diseases (STD) in ethiology of upper urinary tract infections. This study was performed to prove the role of STD in ethiology of urinary tract infections.
Methods: Experimental model of acute pyelonephritis was created on 13 rabbits by ligation of right ureter near urinary bladder with further injecting in ureter cultures of *Ureaplasma urealyticum*, *Chlamydia trachomatis*, *E. coli*, isolated and in different combinations. The final testing was performed on 3 and 7 days.
Results: Inflammation process in upper urinary tract of experimental animals caused by *Ureaplasma urealyticum* has the following pathologic characteristics: the presenñe of ballooning degeneration of pelvic epithelium developed in early stages, the absence of intensive granulocytic reaction. *Ureaplasma urealyticum* realizes its pathogenic properties through membrane destruction with further formation of cell vacuoles and then colliquative necrosis.
The action of *Ureaplasma urealyticum* in combination with non-specific agents (*E. coli*) leads to intensive cell permeability and to early and more intensive developing pyoinflammation reaction. The action of *Chlamydia trachomatis* infection leads to formation of multiple calcified focuses whether its combination with *E. coli* – to primary chronical process formation.
Conclusion: Inflammatory process of upper urinary tract of experimental animals caused by specific agents (*Ureaplasma urealyticum* and *Chlamydia trachomatis*) in case of isolating injection leads to primary chronical process and in combination with non-specific agents to early and more intensive pyoinflammation reaction in the animal's kidney.

P1931 In the bovine trichomoniasis experimental model, systemic immunisation, unlike preputial infection, induces protection against trichomonad colonisation in bulls

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Trichomonads cause human and bovine sexually-transmitted infections, with adverse outcomes of pregnancy in both host species. Bovine trichomoniasis is a useful model because it resembles human trichomoniasis in many ways. Like *Trichomonas vaginalis*, *Tritrichomonas foetus* (*T. foetus*) is an extracellular obligate protozoon that persistently and asymptomatically colonizes the genital tract in males although trichomonad antigens are detected in the preputial mucosal epithelium. We described a preputial "silent" mode of responsiveness in bulls infected with *T. foetus* where males did not mount either a detectable preputial IgA, IgG, IgM or IgE antibody response or increase in immune-inflammatory cells, including mast cells, B, and T cells, and had a slightly increased tissue expression of TGF-beta. Only some *T. foetus* infected bulls showed eosinophilia in the preputial submucosa. However, this "sleeping beauty" mode of responsiveness could be awakened since bulls systemically vaccinated and later challenged with *T. foetus* were capable of resisting trichomonad mucosal colonisation by developing systemic and preputial *T. foetus* specific IgG1 and IgG2 responses. This IgG response was accompanied by an increased population of preputial submucosal HM57+B cells that may produce a portion of the IgG antibodies. In addition, mast cells in the submucosa may assist in transport of IgG from serum to genital lumen by releasing vasoactive inflammatory mediators. Also, in vaccinated and challenged bulls, an increased population of CD3+ T cells and a slightly increased expression of IL-5 in preputial mucosa were observed, suggesting amplified antigen processing and consequently a more productive immune response. Defining mechanisms to explain why the bovine male genital mucosa responds to *T. foetus* "silently" during infection but with an effective local and systemic IgG response after systemic immunisation may contribute to understanding the role of *Trichomonas vaginalis* in men.

P1932 A new animal model for severe complications in intra-abdominal chronic infections caused by *B. fragilis*

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Objectives: Female reproductive organs are often affected by severe complications following intra-abdominal infections (IAI). Such complications, including uterine tube infections and tubulo-ovarian abscesses, can lead to infertility. A new model of chronic uterus infection using a clinical *Bacteroides fragilis* isolate was developed. The model was used to evaluate the efficacy of two antimicrobial agents, moxifloxacin

(MXF) and piperazillin/tazobactam (TZP), which are both approved for the treatment of cIAI in the USA.
Methods: C57BL/6JOla mice were given beta-estradiol 20 mg/kg s.c., followed by beta-estradiol (40 μg/mL) supplied via drinking water. After 4 days, the left uterine tube was surgically prepared and injected with 50 μl *B. fragilis* O6688 (MXF=0.25 μg/mL; TZP = 0.5 μg/mL) suspension (3 x 10^8 colony forming units [CFU] per mouse). CFU in the uterine tubes were determined over 25 days by plating after hysterectomy. Abscess formation was evaluated.
The therapeutic efficacies of MXF and TZP were evaluated using the same infection procedure. Infected mice were treated from day 21 post-infection (p.i.) with MXF 100 mg/kg b.i.d. IV or TZP, 400 mg/kg b.i.d. for 4 days. These doses represent the pharmacokinetics (PK) of human dosages of 400 mg once daily MXF IV and 3.375 g q.i.d. TZP IV therapy, respectively. Efficacy was assessed as the reduction in CFU in the uterine tube homogenates at day 25 p.i.
Results: A chronic *B. fragilis* uterine tube infection was successfully established. Bacterial load in the uterine tube homogenates was stable over 25 days. Abscess formation was first detectable on day 21 p.i. (two out of three mice).
MXF 100 mg/kg b.i.d. achieved a CFU reduction of >–4 logs (99.99%) whilst TZP 400 mg/kg b.i.d. achieved a reduction in CFU of approximately >–2 logs (99%) compared with control (for both P = 0.008, Mann-Witney test). Overall, MXF achieved a significantly higher reduction in CFU than TZP (P = 0.008, Mann–Witney test).
Conclusions: Pre-treatment of mice with beta-estradiol and infection of the uterine tube by surgical preparation allows chronic colonisation of the uterine tube by *B. fragilis* and subsequent abscesses formation. MXF shows superior efficacy to TZP in this model of uterine infection, at doses equivalent to those used in humans.

P1933 Ceftobiprole is superior to cefepime against a *Klebsiella pneumoniae* strain in experimental meningitis

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Objectives: In the present study we tested ceftobiprole (CFT) and cefepime (CPM) as monotherapy against a *Klebsiella pneumoniae* strain in the rabbit meningitis model and determined the penetration of CFT into inflamed meninges.
Methods: We used the rabbit meningitis model as described by Dacey and Sande. The *Klebsiella pneumoniae* strain has been kindly provided by Dr. S. Droz, Inst. Inf. Dis., Bern. The MICs (mg/L) were the following: CPM: 0.25 mg/L, CFT: 0.25 mg/L. Meningitis was induced by intracisternal inoculation of 106 CFU. 8 hours later treatment was started for 8 hours. CFT (40 mg/kg) was injected once at h 0 in order to mimic kinetics in humans. CPM (100 mg/kg) was injected at hour 0 and 4, as a standard dose previously used in this model and corresponding to high doses in humans. CSF samples were repeatedly collected during therapy in order to determine killing rates. Results of the bactericidal activity are expressed in delta log10 CFU/ml·h and delta log10 CFU/ml over 8 h. Penetration of CFT into inflamed meninges was measured by comparison of CSF AUC/serum AUC (Area Under the Curve).
Results: Killing rates of the different regimens are presented in the table.

Group	Inoculum (log_{10} CFU/ml)	Killing rates/h (delta log_{10} CFU/ml·h)	Killing rates/8 h (delta log_{10} CFU/ml·8 h)
Controls (10)	5.39±0.91	+0.20±0.06	+1.76±0.39
Cefepime (10)	5.90±1.23	−0.54±0.17 (1)	−4.54±1.32 (2)
Ceftobiprole (10)	6.28±0.58	−0.76±0.23 (1)	−5.24±1.05 (2)

(1) P < 0.05, ceftobiprole versus cefepime; (2) P < 0.01, ceftobiprole versus cefepime.

Penetration of ceftobiprole into inflamed meninges: mean: 16.37±5.4%. Min.: 9.9%; Max.: 23%
Conclusions:

1. Ceftobiprole is superior to cefepime in the treatment of meningitis due to *Klebsiella pneumoniae* determined by killing per hour or by killing rate over 8 hours.
2. The penetration of ceftobiprole into inflamed meninges ranged around 16%.

P1934 Oral administration of a genetically-labelled *Enterococcus faecium* strain to pregnant mice leads to its presence in foetal meconium

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Objectives: In a previous study, we observed that some bacteria could be isolated from umbilical chord's blood of healthy neonates and from murine amniotic fluid obtained by caesarean section. This suggested that term foetuses are not completely sterile, and that a prenatal mother-to-child efflux of commensal bacteria may exist. Therefore, presence of bacteria in human and murine meconium was investigated.
Methods: Twenty-one meconium samples were aseptically collected from newborns immediately after labor or programmed elective caesarean and plated onto BHI, VRBA and CNA agar plates, which were aerobically incubated at 37ºC for 24 h. Parallel, the same samples were also cultured on WCh and MRS agar plates, which were incubated anaerobically at 37ºC for 48 h. The isolates were examined to determine cell morphology and Gram-staining reaction. Subsequently, a percentage of the isolates, representative of all the colony and cell morphologies observed were identified.
Parallel, a group of pregnant mice were orally inoculated with a genetically-labelled *E. faecium* strain previously isolated from breast milk of a healthy woman. One day before the predicted labour date, they were submitted to caesarean section to collect meconium samples, which were cultured on MRS agar plates. A number of enterococcal colonies were randomly selected and tested for the genetic label
Results: Most of the identified isolates belonged to the Genuses *Enterococcus*, *Staphylococcus*, *Streptococcus*. The labelled strain could be isolated and PCR-detected from meconium of the inoculated animals obtained by caesarean section one day before the predicted labor date. In contrast, it could not be detected in the samples obtained from a non-inoculated control group.
Conclusion: In this study, bacteria could be isolated from meconium obtained from healthy neonates, which suggests that, contrary to what has been hypothesised up to the present, this biological material may not be completely sterile. Such bacteria could initiate gut colonisation as an adaptation of the foetal gut for life outside the mother. Most of the bacterial species isolated in this study are included among the opportunistic pathogens in neonates suffering from underlying conditions. This negative role probably reflects an easy access of such bacteria to predisposed infants since, in fact, they may be part of their endogenous microbiota even at a foetal stage.

P1935 Chicken model of *Enterococcus faecalis* native-valve endocarditis

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Objectives: Enterococci represent the third most common cause of infective endocarditis (IE) and are responsible for 5% to 20% of all cases, of which *Enterococcus faecalis* accounts for >80%. In parallel, antimicrobial resistance continues to evolve and presents serious challenges in the therapy of invasive enterococcal infections. In one recent series, native-valve IE accounted for 61.3% of 44 patients with enterococcal endocarditis. However, most small-animal models of IE more closely mimic the pathophysiology of prosthetic-valve IE. These models rely on the production of sterile aortic vegetations by the insertion of a polyethylene catheter via the right carotid artery, prior to bacterial challenge, which increases the ability of the bacteria to adhere to and colonise the endocardium and, at the same time, decreases the efficacy of the antimicrobial therapy. Therefore, new in vivo technologies for

screening novel candidate agents to prevent and treat native-valve IE are urgently needed. The objective of the present study was to develop a small-animal (chicken) model of *E. faecalis* native-valve endocarditis.

Methods: *E. faecalis* isolates 20326H2 and HEF5 from native-valve IE in chickens and humans, respectively, were tested. Single-age groups of chickens (5 weeks and 50 weeks of age) were injected intravenously (jugular vein) with 10^9 CFU stationary-phase bacteria or sterile phosphate-buffered saline (control groups). Definite IE was identified pathologically according to the modified Duke criteria by 21 days postinfection (at autopsy).

Results: Both groups were highly susceptible to infection with 20326H2 (90% definite IE cases in each group). By contrast, only the adult group exhibited high susceptibility to infection with HEF5 (80% versus 40% in the juvenile group), although the observed difference only reached borderline significance (one-tailed p value = 0.08, Fisher's exact test).

Conclusion: Overall, our data suggest that the chicken model closely mimics the pathophysiology of enterococcal native-valve IE. This conclusion is supported by the observation that the susceptibility to infection with HEF5 increased with age (i.e., due to degenerative valve disease). The present chicken model is readily adaptable to evaluating antimicrobial efficacy and should prove to be a useful tool in identifying bacterial virulence-related factors involved in adhesion to and colonisation of native valves.

P1937 *Caenorhabditis elegans* food-choice experiment: a rapid way to screen for agr-related *Staphylococcus aureus* toxins

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Objectives: *S.aureus* is a remarkably versatile pathogen, capable of causing a diversity of diseases. *C. elegans* is a bacteria-feeding nematode increasingly used for the screening of microbial pathogenic factors. Recent experiments indicate that *C. elegans* larvae fed on wild type *S.aureus* (RN6390) were progressively killed over 5 days, while only 60% of larvae fed on its agr-negative mutant (RN6911) were killed during the same period of time. As a control, all larvae fed on *Escherichia coli* OP50 survived. Hence, some agr-related products might be involved in the killing.

Most natural environments offer a variety of food sources to animals that inhabit them and therefore, feeding usually involves making choices between foods. Here, we tested whether food-choice could be used as a rapid method to detect agr-related toxic products.

Methods: We used early food-choice (at 4 h and 24 h) to test whether the worms could sense agr-related toxins before being killed. About 20 larvae of *C. elegans* were placed at various positions on a series of plates that contained 0.5cm diameter lawns of *E. coli* OP50, *S.aureus* RN6390 or RN6911. Three plates were prepared, each containing two spots of different bacterial lawns. Three zones were demarcated to put the worms: one between the border of the plate and the first bacterial lawn; the second between the two bacterial lawns and the third between the second lawn and the other border of the plate.

Results: Three kinds of results were obtained. (a) When larvae were deposited in the vicinity of a lawn of *E. coli*, >95% of them roamed toward the lawn within 4 h, and all of them kept dwelling there at 24 h; in contrast, <20% of larvae crept toward a lawn of *S.aureus* after 4 h, and all of them were creeping away from the bacteria at 24 h. (b) When *E. coli* and staphylococci were inoculated equidistantly from the larvae, >80% of worms crawled toward *E. coli* irrespective of the *S.aureus* arg-type. (c) When isogenic agr-positive/negative staphylococci where used instead, >60% of the larvae showed a preference for the defective mutant.

Conclusion: *C. elegans* could rapidly sense the presence of agr-related substances. While the nature of the repelling substance(s) has yet to be determined, the food-choice assay represents a simple and faster assay (4–24 h) than the 5-days long-term survival assay to screen for putative microbial virulence factors.

P1938 Intra-catheter leukocyte stain and culture as a diagnostic tool in an experimental animal model of port-related bloodstream infection

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Objectives: Port-related bloodstream infections (PRBI) are difficult to diagnose without port removal. Quantitative blood cultures (QBC) have become the gold standard method for conservative diagnosis. However, this method is laboring, costly and sometimes insensitive. Intra-catheter leukocytes can be isolated, stained by acridine orange (AO) and cultured. The aim of this study was to analyse the usefulness of this method to perform a rapid diagnose of PRBI in an experimental animal model. Secondary we determined the optimal threshold for intracatheter leukocyte culture (ILC) for the diagnosis of PRBI.

Methods: 32 sheep were implanted with venous access ports. Three days after, a standard *Staphylococcus aureus* inoculum was locked inside the port and allowed to dwell for 24 hours. Blood was drawn through port and a peripheral vein three days post-inoculation. Paired QBC, ILC and AO stain were performed. To obtain the intra-catheter leucocytes, 1mL of the blood sample was layered over 1 ml of the extraction solution in a sterile shell vial tube, and centrifuged at 3500 g for 30 minutes. A cellular monolayer was prepared from 50 microliters of the leukocyte band and slides were stained with AO. A positive result was the detection of one microorganism after observing 20 fields by fluorescence microscopy (470 nm). Fifty microliters from the leucocyte band were inoculated into 2 blood agar plates for the ILC. Any cfu observed in the ILC was considered as a positive culture.

Results: We could obtain paired samples in 28 of the 32 animals; devices were obstructed in 4 animals. QBC met criteria for PRBI in 89% of the animals. ILC culture was positive in 100% of the animals, and we could detect some microorganism in 21.4% of the AO stains. The ROC analysis showed that the best threshold for the ILC was 420 cfu/mL (77% sensitivity and 40% specificity). When the cfu in the ILC were higher than 5000 per mililiter, the AO stain improved its sensitivity to 66%.

Conclusion: The results obtained in our model suggest that ILC could be a valid conservative method to diagnose PRBI using a small amount of port blood (1 ml). The AO stain, although it is a simple and quick test, is not very useful for the diagnosis of PRBI. Limitations of this study are the small sample size and the short period of time studied after inoculation, so more studies are warranted.

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P1939 Factors involved in combined treatment with probiotic and fluorokinolone in *Salmonella typhimurium* murine infection

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The aim was to detect the putative factors influencing the eradication of *Salmonella* Typhimurium (ST) by combined treatment with probiotic L. fermentum ME-3 (ME-3) and ofloxacin (OFX) in a murine model.

Material and Methods: Altogether 54 NIH line mice were investigated. Mice from Gr1, 2 and 3 (n = 12 in each group) were infected with a single dose of ST (105 cfu/ml) intragastrically; mice of Gr2 were treated with OFX (20 mg/kg) intragastrically daily. Mice of Gr3 were administered combined treatment with OFX and ME-3 (108cfu/ml) in the drinking water. The uninfected mice of Gr4 were administered ME-3, while Gr5 served as a control with PBS. The mice were sacrificed on Day 5 and Day 10. Blood, liver, spleen and ileum were seeded onto XLD and MRS media to detect ST. Histological slides were prepared from liver, spleen and ileum. GSSG peroxidase and reductase, the glutathione redox ratio (GSH/GSSG), lipid peroxides (LPO) and cytokines (IL-10, INFã and TNFá) in mucosa of ileum and liver were estimated.

Results: *Salmonella* Typhimurium was found in 4 infected mice from Gr1 and Gr2 (2 from blood, 4 from spleen and 1 from liver). The

combined treatment eradicated ST from all investigated sites. Liver granulomas were found in 8 mice in both Gr1 and 2, while the addition of ME-3 reduced the number of mice with liver granulomas ($p=0.019$). The combined treatment caused significant reduction of LPO in mice of Gr3 as compared to Gr1 in the liver and ileum ($p<0.001$; $p=0.005$, resp.). ST infection equally increased the levels of all investigated cytokines as compared to control group. However, in the combined treatment group only the values of IL-10 significantly ($p=0.002$; 0.001 resp.) exceeded these of TNFá and INFã.

Conclusion: In the experimental ST infection the combined treatment with probiotic L.fermentum ME-3 and OFX improves the eradication of ST from gut and reduces the number of granulomas in the liver of mice. The putative factors comprise the reduction of oxidative stress indices and the increase of the anti-inflammatory cytokine IL-10. This may down-regulate the pro-inflammatory cytokines INFã and TNFá and inhibit the formation of liver granulomas.

P1940 Quorum sensing controlled rhamnolipid production is crucial for *P. aeruginosa*s ability to evade the immune response in a mouse foreign-body infection model

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Objectives: *Pseudomonas aeruginosa* is associated with infections on foreign-body implants. The problem with these infections is that they are not cleared even when treated with high doses of conventional antibiotics. The only option to eradicate *P. aeruginosa* is to remove the infected implant. One particular reason for this is the capability of *P. aeruginosa* to grow as biofilms on foreign-bodies. It is widely accepted that cell-cell communication systems, in a process denoted quorum sensing (QS), enables the bacterium to establish and maintain conditions of chronic infections. *P. aeruginosa* uses the QS systems to control the production and secretion of several virulence factors, including rhamnolipids that induces fast necrosis of host cells. Previous data by us generated under in vitro conditions strongly suggests that QS deficiency in vivo rescues the PMNs from the otherwise necrotic effects caused by rhamnolipid producing biofilms.

In the present study we investigated whether a *P. aeruginosa* mutant (rhlA mutant) (which is unable to produce rhamnolipid) will be cleared rapidly from infected mice as compared to the parent wild-type.

Methods: An intraperitoneal foreign-body infection model was used for this study were implants, pre-colonized with either *P. aeruginosa* wild-type or the rhlA mutant, were inserted in the peritoneal cavity of mice. Each group consisted of 12 mice and the experiment was performed twice (N= 48). Three days post-surgical the implants were removed from the mice, and the number of colony-forming units per implant was determined.

Results: Pooled data from the two identically performed experiments showed that almost all the rhlA mutant colonised implants were cleared, resulting in a median CFU of 0; whereas the median CFU for the wild-type was 280 ($p<0.0001$). This result suggests that the difference in clearance is due to *P. aeruginosa* wild-type elimination of the PMNs with rhamnolipid production as the causative agent. To support this, it was examined if rhamnolipid production is essential for *P. aeruginosa* necrosis of PMNs in an in vitro biofilm system. It was found that a high fraction of PMNs became necrotic, when exposed to five days old biofilms of the wild-type *P. aeruginosa*. In contrast, no necrotic PMNs were observed when exposed to biofilms formed by the rhlA mutant.

Conclusion: The present data demonstrates that mutation in a single QS controlled gene (rhlA) impairs *P. aeruginosa* in the battle against the host immune defences.

Tuberculosis: molecular microbiology and diagnosis

P1941 Prevalence of embB306 mutations among ethambutol-resistant and susceptible isolates: correlation with ethambutol and multidrug resistance

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Objectives: Mutations occurring at codon 306 of the embB gene have been extensively reported as the main mechanism by which resistance to ethambutol is acquired in *Mycobacterium tuberculosis* (*M. tuberculosis*). Nevertheless, in the last years several contradictory reports have challenged the role of these particular mutations. The objectives of this study were to analyse the presence of embB306 mutations in both ethambutol-susceptible and -resistant clinical isolates and, to investigate a possible correlation between the presence of embB306 mutations and multi or polidrug resistance.

Methods: We have characterised, by sequencing analysis, a 417bp region of embB gene encompassing codon 306 of 115 *M. tuberculosis* clinical isolates: 66 ethambutol-resistant and 49 ethambutol-susceptible. We have also tested the minimal inhibitory concentration (MIC) for some of these isolates. We have selected ethambutol-resistant isolates in vitro, derived from ethambutol-susceptible H37Rv and clinical *M. tuberculosis* strains, by plating in ethambutol-containing medium.

Results: We have observed that 39 (59%) of the ethambutol-resistant isolates had mutations at codon 306. Eight of the 49 ethambutol-susceptible isolates also had mutations at codon 306 (M306V). The MIC for one of these isolates was shown to be less than 3.75 ug/ml. However, in the in vitro selection for ethambutol resistance, we have obtained two spontaneous mutants with M306V mutation.

Conclusion: We conclude that there is a high prevalence of embB306 mutations among ethambutol-resistant isolates in the Portuguese setting and, no correlation with multi or polidrug resistance was encountered. However, mutations in this codon were also found in susceptible isolates, one of which had a low MIC for ethambutol. This fact is in agreement with previous reports stating that embB306 mutations do not cause resistance to ethambutol. Nevertheless, we have isolated two ethambutol-selected mutants, both bearing a M306V mutation. This is the first report on the isolation of such mutants, which highlights the need of additional studies on this matter.

P1942 N-acetyltranserase 2 gene mutations and bioavailability of isoniazid in tuberculosis patients

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Isoniazid (INH) is a most important drug used in the treatment of tuberculosis. INH is metabolised by N-acetyltransferase type 2 in the liver. The genetically polymorphic NAT2 is responsible for INH metabolism and bioavailability to the blood. Individuals can be classified as rapid or slow acetylators. The acetylation polymorphism is associated with an increased risk of drug toxicity and with and increased frequency of certain cancers. The objective of the study was associations between NAT2 genotyping and INH concentrations in fast and slow acetylators for personalised therapeutic dose.

Methods: Blood samples were taken from 22 patients before (time 0) and 1, 3, 6 hrs after drug administration. Plasma concentrations of INH were determined with biological and chromatography (HPLC) method. In both methods lowest measurable concentration was 0.2 µg /ml. This methods guarantee high accuracy and secured repeatable results. Genotyping: Genomic DNA was extracted by Blood DNA kit and amplified by PCR with two primers: NAT1 and NAT2. After amplification, the PCR product was cut separately with 3 different restriction enzymes: Kpn1, Tag1, and BamH1. A loss of a Kpn1 restriction site denotes NAT2*5 allele, a Tag1 restriction site denotes NAT2*6 allele, and a BamH1 restriction site denotes NAT2*7 allele. The products was separated on agarose gels. The

presence of any 2 mutant alleles defines the slow-acetylator genotype, whereas the rapid acetylators have 1 or 2 wild-type NAT2*4 alleles.
Results: Four different NAT 2 alleles were detected in the tested population. There were 7 different NAT2 genotypes in 22 tuberculosis patients, including *4/*4, *4/*5, *4/*6, *4/*7, *5/*5, *5/*6, *6/*6. INH mean plasma concentration of 9 fast acetylators tested by both methods in 1 h were 2.7 (0.5–5) μg/ml whereas of 13 slow acetylator 4.5 (2–8) μg/ml. For all treated patients concentrations of INH and AUCtotal observed in the rapid acetylators were considerably lower then those found in the slow acetylators $p < 0.05$. The type of mutations in N-acetylotranserase gene were correlated with plasma concentration of INH.
Conclusion: On the basis of our results we suggest the using of NAT 2 genotyping for discrimination of the fast and slow acetylators in monitoring of tuberculosis therapy in one sample of the blood.
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P1943 Expression of phagocyte Fcg receptors during anti-tuberculous treatment

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Receptors for IgG (FcgRs) on phagocytic cells are important in host defence against infection.
Objectives: We have studied the expression of FcgRs by peripheral blood monocytes (M), M cultured for 72 hours (M/Mø), and granulocytes (G) in patients with active Tuberculosis (TB), during anti-TB therapy (anti-TB-Rx) and, after completition of anti-TB-Rx.
Methods: The expression of the three type of FcgRs, FcgRI, FcgRII and FcgRIII, on M, M/Mø and G were analysed by flow cytometry in 149 HIV-negative patients with TB (107 men and 42 women), at diagnosis, and monthly thereafter until completition of anti-TB-Rx. FcgRs expression was assessed on resting M, M/Mø and G, and on these cells after stimulation by culture with IFNg.
Results: The expression of FcgRI and FcgRIII by M, M/Mø and G was enhanced in patients with active TB by: 42±4% and 22±2% for M, respectively ($p < 0.001$), 56±6% and 41±4% for M/Mø, respectively ($p < 0.001$) and, 119±9% and 37±3% for G, respectively ($p < 0.001$). The expression of FcgRIIA by M, M/Mø and G was decreased by −31±1% ($p = 0.02$), −46±3% ($p < 0.001$), and −23±1% ($p = 0.002$), respectively. These alterations of FcgRs expression normalised from the 8th week until the end of effective anti-TB-Rx. The expression of FcgRI, FcgRIIA and FcgRIII by M, M/Mø or G from patients with active TB was significantly increased by culture in the presence of IFNg ($p < 0.001$), returning to normal after 8 wks of anti-TB-Rx. Setting a cut-off value =25% of the mean fluorescence intensity over controls for FcgRs surface expression and, assuming a prevalence range of active TB between 25 and 80% among patients undergoing confirmatory tests, results in a range of sensitivity, specificity, positive and, negative predictive values of: 57%-96%, 48%-97%, 34%-74%, and 59%-98%, respectively for M-FcgRIIA, 48%-73%, 51%-96%, 38%-74% and 68%-97%, respectively for M-FcgRIII, 31%-58%, 64%-93%, 37%-79% and 69%-95%, respectively for G-FcgRI and, 52%-71%, 88%-98%, 49%-77% and 81%-93%, respectively for G-FcgRIIB.
Conclusions: Mø and G from patients with active TB exhibit an altered expression of FcgRs that disappear after effective anti-TB-Rx. Thus, Mø and G FcgRs expression may help in predicting the response to anti-TB therapy.

P1944 Detection of ESAT-6-specific T-cell responses induced by vaccination mice with the preparation KpONE6

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Objectives: The high incidence of tuberculosis (TB) around the world and the inability of BCG to protect certain populations clearly indicate that an improved vaccine against TB is needed. The strategy of using the pathogen itself has given way to creating alternate forms of antigens, new adjuvants and new delivery systems. Our study focuses on delineation possible role polysaccharide matrix and ESAT-6 antigen in modulation of the host immune response. ESAT-6 from *M. tuberculosis* is an important T-cell antigen for cell-mediated immunity.
Methods: To construct plasmid pONE6 the esxA gene was cloned into the eukaryotic expression vector, pcDNA3.1mychislacZ(–) (Invitrogen, USA). To prepare polysaccharide conjugate we modified polyglucin with NaIO4 and subsequent mixing with spermidine. To construct the preparation KpONE6 polysaccharide conjugate and plasmid pONE6 were mixed together. BALB/c mice were immunised on days 1, 14 and 28 with 50 μg of the experimental preparation KpONE6. Cellular responses in the spleen were characterised using ELISPOT, cytokine and proliferation assays. Results were expressed as mean (±)SD.
Results: The preparation KpONE6 was constructed, using DNA plasmid pONE6 and polysaccharide conjugate, to form preparation capable of expressing ESAT-6. It has been demonstrated that the DNA plasmid was protected by polysaccharide conjugate from action of DNase. The experimental preparation KpONE6 was not toxic. We have assessed humoral and cell immune responses in immunised mice. Immunisation with construction KpONE6 no triggers the production of specific antibodies in mice. The IFN-g ELISPOT assay was used to monitor Tcell responses to recombinant antigen ESAT-6. We have reported high levels of antigen-specific IFN-g secretion in mice immunised by KpONE6. Responses to ESAT-6 were significantly higher in the experimental group during the 1st and 2nd immunisations compare with control. Furthermore, we have evaluated cytokine (IFN-g and IL10) production induced by immunisation mice with KpONE6. We have reported high level of IFN-g and low level of IL10 after immunisation by the preparation KpONE6.
Conclusion: So, we have demonstrated that immunisation with preparation KpONE6 induced a Th1-type immune response in immunised animals. With further study and improvement, use of the construction KpONE6 might be a potential vaccine strategy against TB.

P1945 Delayed growth of *M. tuberculosis* and *M. avium* in liquid media after addition of saponin, polypropylene glycol and sodium polyanetholesulphonate

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Objective: The ISOLATOR 10 lysis-centrifugation system (Wampole Laboratories, Cranbury, N.J.) is a technique with excellent results in the recovery of mycobacteria from blood specimens. This system contains saponin (SAP), polypropylenglycol (PPG), and sodium polyanethol-sulphonate (SPS). This study is aimed at determining the effect of SAP, PPG and SPS onto the *Mycobacterium tuberculosis* (4 strains) and *Mycobacterium avium* (4 strains) growth in MGIT and Septi-Chek AFB liquid media.
Methods: SAP, PPG, and SPS were prepared at different concentrations and 0.1 ml of each was added (individually, in pairs, and all of three mixed) to MGIT and Septi-Chek AFB liquid media. Then, liquid culture media were individually inoculated by using two different concentrations (1000 and 100000 CFU/ml) of each of the eight mycobacterial strains used in this study. The media were incubated at 37ºC and its growth was daily surveilled and monitored.
Results: Mycobacterial growth was not prevented but delayed (longer to detect the growth with regard to positive control) by SAP, PPG, and SPS. This is why three substances concentrations delaying mycobacteria growth range from 14–28 mg/ml, 4–8 ml/l, and 10–15 g/l, respectively.
Conclusion: *Mycobacterium tuberculosis* and *Mycobacterium avium* growth in concentrations 1000 and 100000 CFU/ml was delayed by three substances when added alone or in mixture onto MGIT and Septi-Chek AFB liquid culture media. These results suggest it is necessary to propose strategies in order to reduce SAP, PPG, and SPS concentrations which are present in sediment of blood processed by ISOLATOR 10 lysis-centrifugation system, that are finally going to be inoculated in MGIT and Septi-Chek AFB liquid media.

P1946 The diagnostic performance of an interferon-gamma assay in HIV- and tuberculosis endemic population

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Objectives: Pulmonary tuberculosis (PTB) in HIV positive patients is often microscopy negative and the *Mycobacterium tuberculosis* specific Interferon Gamma Release Assays (IGRAs) may be useful in this situation. The performance of IGRAs however, has not been sufficiently documented in tuberculosis- and HIV-endemic settings. We conducted a cross-sectional study in Mwanza, northern Tanzania in order to evaluate the sensitivity of the QuantiFERON TB-Gold In-Tube (QFT-G IT) test for diagnosing pulmonary tuberculosis disease and evaluate the degree to which the test is affected by HIV-status and CD4 cell count.

Methods: 287 patients diagnosed with pulmonary tuberculosis were included in the study and were subjected to control sputum microscopy and culture, HIV- and QFT-G IT testing and measurement of CD4 cell count.

Results: Among 193 patients with culture or microscopy positive TB, sensitivity of the QFT-G IT was 71% (95% CI: 65–77%) and the test was positive in a larger proportion of sputum positive than sputum negative subjects (72% vs. 48%, $p < 0.0001$). Sensitivity of QFT-IT was higher in HIV uninfected than in infected subjects (78% vs. 62%, $p < 0.01$).

56 out of 287 subjects (19.5%) had an indeterminate response due to low mitogen response. The proportion of subjects with an indeterminate QFT-G IT result was larger in those with a CD4 cell count below compared to above 200×10^6/l (38.1% vs. 14.3%, $p < 0.0001$). No other differences were found between patients with determinate and indeterminate responses.

In the 157 subjects with a CD4 cell count above 200×10^6/l, no difference in sensitivity was found between HIV-uninfected and -infected subjects (76% vs. 64%, $p < 0.11$), and the overall sensitivity was not higher (71% vs. 72%, $p < 0.84$). Conversely, the sensitivity was higher when correcting for QFT-G IT indeterminate results (71% vs. 84%, $p < 0.005$).

Conclusion: The sensitivity of the QFT-G IT in this study was similar to previous reports of sputum microscopy sensitivity. The test was affected by low CD4 cell count however this did not explain all the indeterminate results, nor did it affect the overall sensitivity. Other factors than the absolute number of circulating CD4 positive lymphocytes is suggested to influence the performance of the test.

P1947 Is interferon-gamma TB-testing in accordance with guidelines

C.N. Meyer, A. Browatzki (Roskilde, DK)

Objectives: The purpose of this study was to outline the practical use and clinical value of Quantiferon-TB-Gold (QFT-G) testing in the immunodiagnosis of *M. tuberculosis* infection. The main indication for the test is diagnosing latent *M. tuberculosis* infection.

Methods: A retrospective study of all patients tested for *M. tuberculosis* infection by the QFT-G test (Celletis International, Australia) from the 1st of January 2005 to the 31st of December 2006 in a Danish regional hospital.

Results: The QFT-G test was performed in 91 patients. With the intention of diagnosing active TB ($n = 69$), the sensitivity was 80% (8/10, CI=0.55–1.00), specificity was 85% (50/59), PPV was 47% (8/17), and NPV was 96% (50/52). Thus, the positive likelihood ratio (LR) was 5.3 (95% CI 2.7–10.4), and the negative LR was 0.24 (95% CI 0.07–0.8).

The doctors prescribing the QFT-G test were specialists of internal or respiratory medicine (58%, 43/74), infectious diseases (11%, 8/74), haematology (1%, 1/74), dermatology (3%, 2/74), or were junior doctors (13%, 10/74). In 13% (10/74), the prescribing doctor could not be identified.

With a suboptimal diagnostic setup, the diagnosis of lung cancer was delayed three months in one patient with a positive QFT-G. For LTBI, the QFT-G test's approximated sensitivity was 33% (1/3), specificity was 93% (13/14), PPV was 50% (1/2), and the NPV was 87% (13/15), when QFT-G test and Tuberculin Skin Tests were used with the intention of diagnosing LTBI ($n = 17$) where no gold-standard exists.

Conclusion: The QFT-G test had a relatively low sensitivity and a low accuracy in diagnosing active TB, but it was widely used for that purpose. This was contrasting most international guidelines. As most of the prescribing doctors were specialists, it can be assumed that a great deal of uncertainty surrounds the indications for using this relatively new test.

P1948 Tuberculosis in Buenos Aires: ten-month analysis at a reference hospital

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Objectives: (1) Assess the molecular epidemiology of *Mycobacterium tuberculosis* at an infectious disease reference hospital in Argentina. (2) Investigate the prevalence of drug resistance and the underlying genetic background for this.

Methods: 157 patients were enrolled, 57 multi drug resistant (MDR) and 100 fully susceptible. Spoligotyping, VNTR 15 (Variable Number Tandem Repeats), VNTR 7, SNP (Silent Nucleotide Polymorphism) detection using macroarrays and rpoB gene sequencing were performed. Clinical data were collected using a structured questionnaire.

Results: The 157 patients were mainly argentines that lived either in Buenos Aires City or in Greater Buenos Aires; 33% were HIV (human immunodeficiency virus) positive. 62% presented with pulmonary tuberculosis (TB), 80% had a positive smear and 86% of the chest x-rays were abnormal. 18 spoligo families were found. 84% of the 157 strains were clustered in 7 families. When combined with VNTR 15 results, 32% of the strains remained clustered in 3 groups. After adding VNTR data, 2 clusters survived with 12% and 5% of the strains respectively. The 57 MDR strains belonged to 25 HIV positive patients, 25 HIV negative ones and 7 with unknown serologic status. They belonged to 13 spoligo families, 75% were katG mutants whereas 9% were inh mutants. 56 out of 57 had an rpoB mutation. The number of drugs to which bacteria were resistant ranged from 2 to 10.

Conclusions: MDR TB at the Muñiz hospital was mainly represented by 4 spoligo families, with the Haarlem 2 family significantly related to resistance. There was a correlation between the number of drugs to which the strain was resistant and the number of treatments taken showing that compliance is still a major problem in the TB patient management at this hospital.

P1949 Role in quinolone resistance of the new DNA gyrase mutations, T73A+A83E in GyrA and R411H in GyrB, found in clinical MDR and XDR strains of *M. tuberculosis*

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Objectives: Rapid detection of fluoroquinolone (FQ) resistance in multidrug-resistance tuberculosis (MDR-TB) is crucial since ultraresistant TB (XDR-TB, defined as resistant to rifamipicin, isoniazid, FQ, aminosides) is emerging as warned by WHO. Indeed, XDR-TB, which represents 10% of the MDR-TB, are nearly untreatable. Molecular detection of mutations in the DNA gyrase (sole target of FQ in *M. tuberculosis*) could allow the rapid detection of resistance to FQ in *M. tuberculosis*. However, all new mutations found in clinical strains should be evaluated for their implication in FQ resistance. In this work, we investigated the role of two new mutations (T73A+A83E in GyrA and R411H in GyrB) found in DNA gyrases of MDR and XDR clinical strains in resistance to FQ in *M. tuberculosis*.

Methods: Four mutant genes, reproducing the mutations (T73A+A83E in GyrA and R411H in GyrB) found in clinical strains, as well as the two single mutations (T73A or A83E), were produced by site-directed mutagenesis of the wild type gyrA or gyrB genes previously cloned in pET vectors. WT and mutant GyrA and GyrB subunits were

overexpressed in *E. coli*, purified and used to reconstitute highly active gyrase complex. Enzyme inhibition (concentration of drug required to inhibit the supercoiling activity of the enzyme by 50% = IC50) were determined for moxifloxacin, gatifloxacin, ofloxacin and garenoxacin.
Results: IC50s of FQ were identical for the WT enzyme and the mutant gyrase carrying the GyrB R411H substitution, demonstrating that this mutation is not implicated per se in FQ resistance. Interestingly, gyrase complexes bearing GyrA T73A were hypersusceptible to FQ as previously shown (IC50s at least 2 fold lower than the WT IC50s), whereas those bearing GyrA A83E were highly resistant to FQ (IC50s 15 to 25 fold higher than the WT IC50s), an effect that was attenuated for enzymes bearing both mutations (IC50s 3 to 4 fold higher than the WT IC50s).
Conclusion: We demonstrated unequivocally (a) the implication of the single mutation A83E and the double mutation T73A+A83E in GyrA in resistance to FQ in *M. tuberculosis*., and (b) the non implication of the mutation R411H in GyrB in FQ resistance in *M. tuberculosis*. These results underlies (a) the importance of demonstrating the role of each new DNA gyrase mutation in FQ resistance, and (b) the occurrence of mutations leading to hypersusceptibility and attenuating the level of resistance of associated mutations.

P1950 In vitro selected mutants of *M. tuberculosis* do not reflect the in vivo mechanism of isoniazid resistance

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Background: Multidrug-resistant tuberculosis (MDR-TB) is by definition resistant to at least isoniazid (INH) and rifampicin (RIF), the two most commonly used drugs against *M. tuberculosis* (MTB). Monoresistance to INH is much more common in clinical isolates than monoresistance to RIF. In addition, the in vitro rate of INH resistance is typically hundreds of times higher than the rate for RIF resistance. It is generally assumed that the high in vitro mutation frequencies explain the high prevalence of INH resistance in vivo.
We studied the in vitro mechanisms of INH resistance, in part to determine the preferred genetic routes to MDR under various conditions. Here we discuss our findings on INH resistance mechanisms in vitro.
Methods: We determined the frequencies and rates of spontaneous mutations conferring INH resistance under various conditions, using well-characterised MTB laboratory strains. Mutation frequencies were determined by counting the proportion of resistant mutants on solid medium and mutation rates were determined by Luria and Delbrück fluctuation assays (po-method). For each experiment and condition INH-resistant mutants were picked randomly and characterised by PCR, sequencing or MLPA.
Results: We found that the INH resistance rates and frequencies were approximately two logs higher (10^{-6}) than those previously measured for RIF resistance (10^{-8}). However, the katG gene was partially or completely deleted in the majority (30–90%, depending on the strain and condition) of the INH-resistant mutants. In contrast, INH-resistance in vivo is almost always due to point mutations in katG-315 or inhA(−15). Attempts to select against katG deletion mutants, in favour of clinically relevant INH-resistant mutants, by selecting under oxidative conditions, were unsuccessful.
Conclusion: The high prevalence of INH-resistance in vivo has been attributed to the high rate of mutations conferring resistance to this drug in vitro. Based on our results and the scarce literature available, we feel that this is at best a simplification as the majority of the in vitro mutations are rarely seen in clinical isolates. Moreover, KatG is the only active defence against oxidative assault in MTB, but the prevalence of katG deletion mutants was not significantly reduced under oxidative conditions.
These results indicate that additional research into the emergence of INH resistance is needed and that assumptions based on the observed INH resistance rates in vitro may be unwarranted.

P1951 Diagnosis of *Mycobacterium tuberculosis* from extrapulmonary specimens with real-time PCR

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Objectives: Tuberculosis remains a public health problem in Portugal. Rapid detection of *Mycobacterium tuberculosis* plays a key role in control of infection. Rapid diagnosis of extrapulmonary tuberculosis has a greater impact on patients management than on limiting spread of the disease.
Our objective was to evaluate the routine use of Real Time PCR for recovery of *M. tuberculosis* from extrapulmonary specimens and to compare the results with culture.
Methods: A total of 193 (82 LCR and 111 other extrapulmonary specimens) were tested using artus *M. tuberculosis* RG PCR Kit. This method consists of a system for detection of DNA of all members of the *M. tuberculosis* complex, that amplified a specific 159 bp region of mycobacterial genome. The amplified products were detected by fluorescent dyes. The same specimens were inoculated in the solid medium Lowenstein Jensen (LJ) and in the liquid media MGIT (Mycobacteria Grwth Indicator Tube).
Results: Of the 193 specimens 15 (7.8%) were positive on Real Time PCR, being 12 LCR and 3 other extrapulmonary specimens. All LCR samples were negative on culture. The PCR positive extrapulmonary samples (synovial fluid, knee biopsy and purulent fluid) were also positive on cultures in both systems LJ and MGIT.
All positive PCR results were confirmed by clinical diagnosis.
Conclusion: Real Time PCR is a sensitive and specific method which permits a rapid diagnosis of tuberculosis in particular cases of paucity bacillary specimens. The application of the technique to extrapulmonary samples, although not recommended by manufactures is an important field. In this work we demonstrate the useful application of a real time PCR kit to extrapulmonary samples, particullary to LCR with a very good sensitivity.

P1952 QuantiFERON®-TB Gold test in homeless shelter staff: preliminary results

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Objectives: Healthcare workers are particularly at risk of contracting and/or transmitting tuberculosis (TB), since the spread of the mycobacterium often occurs before the diagnosis of the infection. In healthcare settings, policies and procedures for TB control should be developed, including health surveillance. Recently, the introduction of new in vitro serological tests, as the Quantiferon ®TB-Gold in tube (QFT-TB), could overcome problems associated to the tuberculin skin test.
In this preliminary study 27 nuns (homeless shelter staff) were tested with QFT-TB and were subjected to a questionnaire in order to identify the potential risks of their work-related activities and to propose specific prevention programmes.
Methods: The study was conducted in September 2007. A questionnaire was administered to collect information about age, place of residence, place of work, work-related activities, duration of employment, contacts with *M. tuberculosis*, symptoms compatible with TB, previous TB diagnosis and vaccination state with BCG. The QFT-TB test, performed according to the manufacturer's instructions (Cellestis, Australia), is based on the quantification, by ELISA, of interferon-gamma released from sensitised lymphocytes in whole blood incubated overnight with PPD from *M. tuberculosis* and control antigens.
Results: The mean age of the nuns was 45.51 ± 11.75. Fifhteen out of 27 samples were positive (55.5%); one plasma sample (3.70%) was indeterminate and the remaining 11 (40.74%) were negative. The indeterminate result was probably due to the low response of stimulated T-cells to mitogen (<0.5IU/ml). Data from questionnaires were evaluated for possible risk factors. Among the 14 nuns coming from countries endemic for TB, 10 were positive to QFT-TB test. Moreover, other 4 positive subjects had only previously worked in countries endemic for

TB. Work-related activities of nuns were classified in 4 groups: homeless shelter staff involved in the first assistance of the guests (62.9%), nurses (14.8%), office staff (11.1%) and food service staff (3.7%); the first group showed the highest positivity to the QFT-TB test.
Conclusion: This study showed that the high prevalence of positive tests was associated with risk factors such as the places of residence and of work. Moreover, work-related activities, duration of employment and contacts with *M. tuberculosis* were also identified as contributing to the increased level of exposure to mycobacterium.

P1953 Quantiferon-TB: a new test for diagnosing tuberculous uveitis

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Purpose: Tuberculose skin test (PPD) has limited power for diagnosing tuberculosis since active disease cannot be differentiated from immunity. Therefore, we evaluated the usefulness of a whole-blood interferon-g enzyme-linked immunosorbent assay (QuantiFERON TB-2G [QFT]; Cellestis) in obtaining a diagnosis of tuberculosis in patients with uveitis (TB).
Methods: QFT-G is a whole blood, antigen-specific, test that utilizes synthetic peptides representing two *M. tuberculosis* proteins, ESAT-6 and CFP-10. After incubation for 16 to 24 hours, the amount of interferon-g secreted by T lymphocytes in response to these antigens is measured. We used the test in 34 uveitis patients in which tuberculosis was included in the differential diagnosis. These were patients with either active granulomatous uveitis unresponsive to topical or systemic corticosteroid therapy (n = 12), or inactive posterior uveitis with a serpiginous/multifocal chorioiditis (MCP)-like presentation (n = 15), or posterior uveitis with different clinical pictures (n = 6). These rates were compared to a group of healthy hospital personnel.
Results: 15/34 patients (44%) were tested positive. Of the patients with active inflammatory disease 4 were tested positive (33%). 60% (9/15) of our patients with inactive, posterior, serpiginous like uveitis were tested QFT-positive. Only 1 patient (17%) of the patients with posterior uveitis of different presentation was QFT positive. The rate of QFT-positivity in the healthy control group (n = 208) was 6%.
Conclusions: TB-uveitis seems to present in two forms: active granulomatous disease and inactive disease with chorioretinal scarring like serpiginous choroiditis or MCP. Admitting patients with the latter form to QFT testing increases the post-test-probability of TB. QFT testing seems to be a useful test in the differential diagnosis of tuberculous uveitis.

P1954 Evaluation of QuantiFERON TB gold assay in tuberculosis diagnosis

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Objective: To evaluate QuantiFERON TB Gold assay (QF), in respect to agreement with Tuberculin Skin Test (TST), BCG vaccination, immunosuppresion, exposure to *M. tuberculosis* (MTB) and active TB.
Materials and Method: QF was performed in 480 whole blood samples. Clinical data was partially available in many instances. Sputum cultured were 65 patients.
Results:
– In 480 cases studied, QF(+) were 159 (33.1%), QF(–) 298 (62.1%) and indeterminated 23 (4.8%).
– TST data was available in 443 cases: TST(+) were 297 (67.1%) (zone diameter ⩾10 mm) and TST(–) 146 (32.9%). Among TST(+) individuals, QF(+) were 123 (41.4%), QF(–) 172 (57.9%) and indeterminated 2 (0.7%). In TST(–), QF(+) were 28 (19.2%) QF(–) 105 (71.9%) and indeteminated 13 (8.9%) individuals.
– BCG vaccination – TST data was avaible in 161 individuals: 114 (70.8%) were TST(+) QF(–), 45 (27.9%) were TST(+) QF(+) and 2 (1.7%) were TST(–) QF(–).
– In 323 cases, there was sufficient data for immunologic status: 52 were immunosuppressed and 271 immunocompetent. In immunosuppressed, TST(+) were 15 (28.8%) and TST(–) 37 (71.2%), while QF(+) were 17 (32.7%), QF(–) 26 (50%) and indeterminated 9 (17.3%). In imunocompetente, indeterminate results were much lower 6/271 (2.2%).
– MTB exposure – BCG vaccination – TST data was available in 118 cases. Among them, in 48 BCG non vaccinated – MTB exposured individuals, QF(+) was 14 (29.1%), while TST(+) 25 (52%).
– Sputum cultured – QF tested were 65: In 23 positive cultures, 20 MTB and 3 NonTB were isolated. All MTB (+) cases were QF(+), while none in NonTB patients.

Conclusions: 19.2% of TST(–) cases were positively diagnosed for TB by the QF. Indeterminated results were more common in TST(–) as well as in immunosuppressed cases. In BCG vaccinated, QF contributed in diagnosis of 27.9% MTB infections. In immunosuppressed, QF(+) results were 4% higher than TST(+). TST sensitivity was higher than QF in exposured individuals. In BCG vaccinated, exposured, QF contributed in diagnosis MTB infection by 20%. All MTB(+) cultured patients were QF(+).

P1955 The diagnosis of tuberculous meningitis before and after antituberculous treatment using nested PCR

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Objectives: Due to the difficult and tardy bacteriologic confirmation of tuberculous meningitis (TBM), most patients receive antituberculous treatment in urgency, based only on clinical and cerebrospinal fluid (CSF) characteristics. The objective was to establish the efficiency of PCR as a rapid method for the diagnosis of TBM, before an also after antituberculous treatment starting.
Methods: We evaluated the nested PCR technique using primers directed against the IS6110 gene, for the detection of *Mycobacterium tuberculosis* in 91 samples of CSF, collected from 31 patients hospitalised in the Infectious Diseases Institute Prof. Dr. Matei Bals, Bucharest, from December 2006 to September 2007: 15 patients with TBM and 16 patients with nontuberculous meningitis. The CSF samples were collected at admission and on 7, 14, 21, 30 days after antituberculous treatment starting. Clinical, epidemiological dates, CSF modifications, other tuberculous locations and the good response to antituberculous treatment supported the TBM diagnosis. Five patients were bacteriologically confirmed.
Results: Thirteen out of 15 CSF samples were PCR positive on hospital admission and also after 7, 14 and 21 days of treatment and 9 out of 15 CSF samples remained PCR positive after 30 days of treatment. Ziehl Neelsen smear was positive at admission only at one patient and culture on Lowenstein media was positive at 5 out of 15 patients; CSF cytology and biochemical modifications were characteristic at 13 out of 15 patients on admission and also within the following 14 days after treatment. There were two false positive PCR results at patients with bacterial meningitis.
Conclusion: Positive results of nested PCR using IS6110 were observed at most TBM patients on hospital admission and also after a long period of antituberculous treatment, compared with bacteriologic or cytochemic analysis of CSF. PCR method could be helpful in diagnosis of TBM due to its rapidity and sensitivity even after a long period of antituberculous treatment starting.

P1956 Comparison of an interferon-gamma assay with tuberculin skin test for the diagnosis of tuberculosis infection prior to anti-tumour necrosis factor therapy

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Objectives: To compare the tuberculin skin test (TST) with a whole-blood interferon-gamma (IFN-g) assay in the diagnosis of tuberculosis

infection (TBI) in patients with inflammatory diseases due to star anti-tumour necrosis factor (TNF) treatment.

Methods: A multicentre, cross-sectional study of the patients evaluated at 4 Spanish hospitals, from October 2006 to October 2007, before starting anti-TNF therapy. Diagnosis of TBI was based on TB exposure, chest X-ray, two-step TST and IFN-g assay (QuantiFERON® TB Gold-in Tube, QFT). According to the manufacturer's instruction, QFT was considered as positive when the TB antigen minus negative control IFN-g production $\geqslant$0.35 UI/mL; an indeterminate result was defined as either a positive control IFN-g response of $\leqslant$0.5 UI/mL or a negative control IFN-g level of >8 UI/mL. A positive TST was defined as a $\geqslant$5 mm induration. Agreement between TST and QFT was assessed by the Cohen kappa (k) index.

Results: 142 patients were screened for TBI, 51% women, with a mean age of 49 years. 44% had rheumatoid arthritis, 20% spondyloarthropathy, 19% cutaneous psoriasis, 10% psoriatic arthritis, 6% inflammatory bowel disease and 1% Behçet disease. 69% were under immunosuppressive treatment, mainly corticoids (39%) and methotrexate (38%). 23% were BCG vaccinated, 4% had a previous story of TBI and 6% had an abnormal X-ray. 33% of patients had a positive TST compared with 23% positive QFT ($p < 0.01$). Patients with some TB risk factor had 55% positive TST ($p = 0.03$) but only 32% positive QFT ($p = 0.33$). Overall agreement between the two tests was 81% (113/139), (k=0.54, 95% CI 0.38–0.7). Agreement was lower in patients with positive result by one of two tests (50%, $p = 0.04$). Agreement between the two tests was not influenced by BCG status (72% versus 83%, $p = 0.16$) neither by immunosuppressive treatment (81% versus 79%; $p = 0.7$). There were 3 (2%) indeterminate QFT results due to low IFN-g production in positive control.

Conclusions: Overall agreement between TST and QFT was fair, but among those patients with positive result by either of two tests was low. These results question the use of QFT as the only diagnostic test for TBI in this population. The rate of indeterminate results was low.

P1957 Comparison of Two Acid-Fast Microscopy Methods for the Detection of Acid-Fast Bacilli in Sputum Specimen

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Background: The early diagnosis of tuberculosis still depends on the presence of Acid-Fast Bacilli (AFB) in stained smears. For this reason different AFB staining methods are used. In this study we aimed to investigate the efficiencies of a two different fluorochrome stains, and the costs of commercial and in-house prepared stains.

Methods: This study was conducted in a three-month period in 2005. Sputum specimens were obtained from Kahramanlar Region Tuberculosis Laboratory (Izmir/Turkey) where 3420 specimens were accepted for routine AFB detection in the study period. We have chosen 1013 specimens from 642 patients by random sampling. Laboratory procedures were performed as double blinded and prospectively. Three smears were prepared each sputum specimens. Slides were stained with commercial Auramine kit, Auramine/Acridine orange (ST 022, Salubris), in-house prepared fluorochrome stain, Auramine-Rhodamine/KMnO4, and Ehrlich-Ziehl-Neelsen (EZN) methods. All specimens were cultured on Löwenstein-Jensen medium. All of the slides identified as positive by fluorochrome stains were restained with EZN. Specificities, sensitivities, negative and positive predictive values of methods were calculated according to the culture results and cost of fluorochrome staining methods were compared.

Results: Among 1013 specimens, 101 were culture positive. Among these, AFB was detected in 60 specimens by EZN, in 53 specimens by commercial Auramine kit, in 81 specimens by in-house prepared fluorochrome method. Specificities, sensitivities, negative and positive predictive values of methods according to the culture results are shown in table. In cost analyses the cost was found 15, 3 euro/100 slides for commercial Auramine kit, and 17, 8 euro/100 slides for in-house prepared fluorochrome method.

Staining method	Culture results			Sens.	Spec.	PPV	NPV
	Positive	Negative	Total	(%)	(%)	(%)	(%)
In-house prepared Auramine-rhodamine/$KMnO_4$				**80.1**	83.8	35.5	**97.4**
Positive	81	147	228				
Negative	20	765	785				
Total	101	912	1013				
Commercial Auramine/acridine orange				52.4	**94.6**	**42.7**	94.6
Positive	53	71	124				
Negative	48	841	889				
Total	101	912	1013				

Conclusion: The commercial Auramine kit was easy and inexpensive but the sensitivity of Auramine/Acridine orange was lower compared to Auramine-Rhodamine/ KMnO4. The sensitivity of in-house prepared Auramine-Rhodamine/KMnO4 method was higher, but it required longer preparation time, more workload and had more risk for carcinogen exposure. The specificity and positive predictive value of Commercial Auramine/Acridine orange was higher than in-house prepared Auramine-Rhodamine/KMnO4. In order to increase sensitivity when using the commercial kit, it may be recommended that KMnO4 should be preferred instead of acridine orange as the counter stain.

Molecular detection of bacterial species

P1958 Multiplex PCR for specific detection of meticillin-resistant staphylococci in a group of patients with infective endocarditis

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Objectives: Rapid detection of causative pathogen is crucial for successful antibiotic treatment in patients with infective endocarditis (IE). Staphylococci are among the most common aetiological agents in heart valve infections and their resistance to meticillin has become more frequent in last years. However, cultivation (including antibiotic resistance testing) takes several days or remains negative due to prolonged antimicrobial treatment.

The aim of this study was to i) characterize the patients with staphylococcal IE in details, ii) develop an efficient molecular identification procedure specific for staphylococci, and iii) determine meticillin resistance in detected staphylococcal strains.

Methods: Since April 2004 to October 2007 one hundred and eighty four valve samples from patients with suspected IE were collected. Broad-range PCR (br-PCR) followed by direct sequencing were used to detect and identify DNA of responsible pathogens. Out of the positive PCR samples, 51 (42.3%) were identified as staphylococci.

In this study, specific PCR was designed for three target genes with different length of PCR fragments: i) part of the 16SrRNA gene specific for all staphylococci, ii) the femB gene encoding enzyme specific for *Staphylococcus aureus*, and iii) the mecA gene for detection of meticillin-resistance.

Results: Of the 51 patients included into the study 28 were classified as definite IE (55%), 15 as possible IE (29%) and 8 cases did not fulfil the Duke criteria (16%). Six patients were drug addicts with *S. aureus* being the only causative agent found. Prosthetic valves were present in 35 cases (69%).

DNA samples of 45 staphylococcal strains characterised earlier by br-PCR and sequencing were available for multiplex PCR testing. The setting included 31 *S. aureus*, 13 *S. epidermidis* and 1 coagulase negative (CN) strain not identified to the species level. Six samples were excluded for bad DNA quality. The results of multiplex PCR were available within six hours and correlated fully with br-PCR and sequencing. Eleven CN staphylococci but no *S. aureus* showed presence of meticillin-resistance gene.

Conclusion: We developed a fast, sensitive and specific multiplex PCR system for simultaneous identification of *S. aureus*, CN staphylococci

and the mecA gene. We evaluated this technique on heart valve samples in patients with IE. Twenty-four percent (n = 11) of detected staphylococci in valves were meticillin resistant, all of them were coagulase negative.

P1959 Discrepancy analysis of conventional culture and PCR for screening of meticillin-resistant *Staphylococcus aureus*

A.T.A. Box, M.W.M. Wassenberg, A. Troelstra, J.A.J.W. Kluytmans, M.J.M. Bonten on behalf of the Dutch Rapid MRSA Diagnostics Study Group

Objectives: To evaluate discrepancies between MRSA-PCR and conventional cultures which occurred in a prospective multi-centre study on cost-efficacy of adding PCR to the currently used search and destroy policy in the Netherlands.
Methods: The study was performed from 12/05 to 06/07 in 12 hospitals. In all patients screened for MRSA upon admission swabs were taken for PCR (BD GeneOhm MRSA PCR) and conventional culturing including selective enrichment broth (SEB). Cultures were regarded golden standard. In case of discrepancies, swabs were taken for repeated PCR and for selective culturing of MSSA. PCR-lysates and culture-broths were stored for analysis at the end of the study.
Results: From 902 patients, 4 patients (0.4%) had false negative (FN) PCRs. Bacterial load of MRSA presumably was below PCR-detection limit in samples of 2 patients, as MRSA was identified after subculturing of the SEB only. In 2 patients the assay failed to detect pig-related ST398 MRSA, known for its variability in the SCCmec sequence not always allowing amplification with the panel of primers. Thirtyfive patients (3.9%) had FP results: 6 patient samples revealed MSSA (SCCmec-orfX positive; mecA negative); in 3 other patients this was suspected based on characteristics of the PCR-curves; 1 patient was MRSA-positive with an initial bacterial load below the detection limit of culture; in 7 patients FPs were due to PCR contamination, in 3 patients contamination was suspected. The reason for false-positivity remained unknow for 15 patients (43%).
Conclusion: In daily practice, routine screening for MRSA with PCR as compared to conventional culturing with SEB, had a FP rate of 3.9% and a FN rate of 0.4%. FPs mainly resulted from MSSA containing a residual SCCmec right-extremity fragment (26%) and contamination (29%).

P1960 External quality control of the GeneXpert® system for identifying meticillin-resistant *Staphylococcus aureus*

A.T.A. Box, M.W.M. Wassenberg, A. Troelstra, J.A.J.W. Kluytmans, M.J.M. Bonten on behalf of the Dutch Rapid MRSA Diagnostics Study Group

Objectives: To assess the quality of MRSA testing using the Xpert MRSA assay on the GeneXpert® system (Cepheid) in 9 Dutch hospitals.
Methods: In a prospective multi-centre study, which started 04/07 and will last till 04/08, hospitalised patients with a high-risk on MRSA-carriership are screened for MRSA using the Xpert MRSA assay. The sequences targeted by this real-time PCR assay are within the right extremity of the SCCmec cassette and orfX (described by Huletsky). All participating centres performed testing of a blinded quality control panel of 6 swabs containing 840, 160, 84, 8.4 and 0.84 CFU of MRSA-ATCC-43300 per swab and an additional swab containing 160 CFU of MRSA and 48000 CFU of meticillin-susceptible *Staphylococcus epidermidis* (MSSE-ATCC-12228). All cycle-time (Ct) and endpoint values were analysed.
Results: All laboratories found positive results for swabs containing 840 and 160 CFU of MRSA. Only 2 (22%) detected 84 CFU correctly; with Ct-values of 35.2 and 35.3. Five laboratories did not detect 84 CFU of MRSA, but found CT-values ranging from 36.2 tot 38.0; two laboratories found no CT-values. No laboratory detected 8.4 and 0.84 CFU. All labs were able to detect MRSA on the swab loaded with MRSA-MSSE with mean CT-values of 33.6 that were only slightly higher than values for the swab with MRSA only (mean 32.6).
Conclusion: All participating centres performed comparably well in the quality control for 840 and 160 CFU of MRSA per swab. Only 2 out of of 9 of laboratories detected 84 CFU. The detection limit of the PCR in this study was 160 CFU, which is higher than the 50 CF which is reported by the manufacturer. Abundantly present MSSE did not influence detection of MRSA.

P1961 Direct detection of MRSA in surveillance samples by Xpert MRSA

H. De Beenhouwer, G. Duson, J. Frans, A. Van den Abeele, R. Cartuyvels, G. Coppens on behalf of the Bilulu Study Group

Objectives: Evaluation in a multi-centre setting of the performance of a rapid commercial molecular technique (Cepheid's Xpert MRSA assay) for the detection of MRSA in surveillance samples of high-risk patients in comparison to standard culture technique.
Methods: High-risk patients (n = 236) were sampled from April till June 2007 in 5 Belgian hospitals. Separate nasal, throat and perineal swabs were collected using the Cepheid collection device (Double Copan Swab). In addition, swabs were taken according to each specific hospital procedure.
One part of the double Copan nose, throat and perineum swabs was pooled and vortexed in the Elution Reagent and then transferred to the Xpert MRSA cartridge. Molecular testing was performed on the GeneXpert according to the manufacturer's instructions. The system combines extraction, real-time PCR and detection in one hour.
The other part of the double swabs was pooled, frozen and sent to 1 laboratory that performed the reference culture, using TSB with 5% NaCl and subculture after 24 hours on a chromogenic agar plate.
The own procedure swabs (nose, throat and perineum) were cultured in-house, also using selective enrichment and chromogenic agar according to local procedures.
Results: 27 Samples (11.4%) showed inhibition on the GeneXpert and were not included in the analysis.
The Xpert MRSA assay identified 97.4% of the specimens positive for MRSA and 86.5% of the specimens negative for MRSA. For the samples tested, the Positive Predictive Value was 62.3% and the Negative Predictive Value was 99.3%.
The PPV of the GeneXpert results versus the Reference culture and versus the In-house culture method differed significantly (62.3% versus 80.3%). Freezing and thawing might have been injurious to the viability of MRSA. For almost all hospitals, the PPV decreased significantly when performed with the Reference Culture method.

	Ref. culture +	Ref. culture −	Total (N = 236)
GeneXpert +	38	23	61
GeneXpert −	1	147	148
Total	39	170	209
Invalids	1	26	27

Conclusion: The Cepheid's Xpert MRSA assay proves to be an efficient test to rule out MRSA (NPV=99.3%). The test provides rapid results (about one hour) which might be helpful in prevention and control of MRSA in hospitals. However, because of a high rate of 'invalids' and a low PPV, a culture method is still needed to confirm a positive result.

P1962 **Cost-efficacy of rapid diagnostic testing of meticillin-resistant *Staphylococcus aureus* in a low-endemic setting**

M.W.M. Wassenberg, A.T.A. Box, A. Troelstra, J.A.J.W. Kluytmans, M.J.M. Bonten on behalf of the Dutch Rapid MRSA Diagnostics Study Group

Objectives: Pre-emptive isolation of high-risk patients is a cornerstone of the Dutch search and destroy strategy to control MRSA. However, many high-risk patients are not colonised and remain in isolation for 3 to 5 days awaiting conventional culture results. We determined cost-efficacy of addition of rapid diagnostic testing (BD GeneOhm MRSA PCR) to the current prevention policy in a prospective multi-centre study.
Methods: All patients with increased risk of MRSA colonisation and fulfilling the criteria for pre-emptive isolation in 12 participating hospitals between 12/05 and 06/07 were eligible. In addition to the standard set of conventional microbiological cultures, MRSA-PCR was performed directly on patient material. Infection prevention measures were based upon immediate PCR-results, and, thus withdrawn when negative.
Results: 902 patients were enrolled. Mean age was 47 year and 55% were male. Reasons for MRSA screening were hospitalisation abroad (74%), contact screening related to MRSA-positive index patients (15%) and contact with pigs (7%). 3406 PCRs were performed (3.8 per patient including nose, throat, perineum). The MRSA prevalence was 3.0%. Compared to culture sensitivity, specificity, positive predictive value and negative predictive value of the MRSA-PCR were 85.2%, 96.0%, 39.7%, and 99.5%, respectively. In 83% (747/902) of patients isolation was discontinued upon negative PCR-results (4 patients had a false-negative result without any clinical consequences). Times from pre-emptive isolation to notification of PCR and culture results were 21 hours and 97 hours respectively. 26% (193/747) of the patients with negative PCR were already discharged before conventional results were available. Therefore, 1935 isolation days were saved. Costs of the PCR were estimated €52,– per test; therefore costs per isolation day saved were €88,–.
Conclusion: In a low-endemicity setting, guiding of pre-emptive isolation upon MRSA-PCR results is safe and associated with a reduction in isolation time from four to less than one day at the costs of €88,– per isolation day saved.

P1963 **Ultra fast protocol for direct detection of MRSA from swabs**

U. Eigner, A. Fahr, M. Weizenegger (Heidelberg, DE)

Objectives: We modified and validated the protocol of the GenoQuick®MRSA direct version 2 beta (Hain Lifescience, Nehren, Germany) by introduction of a high speed Taq-Polymerase (Speed-STAR™ HS-DNA-Polymerase (TaKaRa, Otsu, Japan). The GenoQuick® MRSA assay is a PCR based assay targeting the staphylococcal cassette (SCCmec). For the detection of the MRSA specific amplicon and the "amplification control" (AC) a lateral flow dipstick is used.
Methods: For nucleic acid based identification of MRSA from swabs the prelaunched GenoQuick MRSA version 2 beta was used. The assay includes a lysis mix for isolation of the staphylococcal DNA, a PCR based amplification mix and a dipstick for detection. 329 DNA isolates from swabs sent for routine diagnostic were investigated. DNA-isolates (frozen at −70ºC for several weeks) were taken from a previous study. The results were compared to routine testing including a chromogenic agar (CHROMagar MRSA, Becton Dickinson, Heidelberg, Germany) and a trypticase/soy broth. Cultured MRSA strains were confirmed with the GenoType MRSA (Hain Lifescience) test.
The PCR protocol recommended by the manufacturer was shortened to 15 min, 95ºC, 40 cycles of 5 sec 95ºC, 10 sec 55ºC, 10 sec 72ºC and for final denaturation 1 cycle of 2 min 95ºC and 5 min 20ºC. Samples run on a PE 2720 thermo cycler (Applied Biosystems, Darmstadt, Germany).
Results: 48 isolates had a congruent positive result and 277 a congruent negative result (sensitivity 96.1%, specificity 99.3%, positive predictive value 96.1% and negative predictive value 99.3%. Two samples of culture positive MRSA were missed. Two specimens had a false positive result with the GenoQuick assay. These two false positives were identified as meticillin (oxacillin) susceptible *Staphylococcus aureus* (MSSA) bearing a SCCmec missing the mecA gene. Six culture negative samples resulted weak positive with the PCR-test. All converted negative when repeated.
Conclusions: The modified GenoQuick®MRSA assay adapted to the SpeedSTARTMHS-DNA-Polymerase shortens the time to result for the detection of MRSA from swabs from 2.5 to 1.5 hours. Together with the quick DNA-isolation procedure and an efficient manual hands on time this assay represents a very time optimised tool for direct detection of MRSA. Compared to the manufacturer's protocol no decline of the sensitivity and specificity values were observed.

P1964 **Rapid identification of clinical bacterial isolates using the Matrix-Assisted Laser Desorption Ionisation-Time of Flight mass spectrometry in a clinical laboratory**

U. Eigner, A. Hendel, U. Wild, F. Wild, M. Holfelder, A. Fahr (Heidelberg, DE)

Introduction: Matrix-Assisted Laser Desorption Ionisation-Time of Flight Mass Spectrometry (MALDI-TOF MS) produces specific mass spectral fingerprints for different micro-organisms and is used for the identification of clinical bacteria.
We performed an evaluation study for the implementation of a MALDI-TOF system (MALDI BioTyper 2.0, Bruker Daltonik GmbH, Bremen, Germany) in the routine bacteriology laboratory. Therefore, the results of the phenotypic bacterial identification systems (VITEK2, api system (bioMérieux, Nürtingen, Germany) in our routine laboratory were compared to MALDI-TOF.
Methods: For the evaluation study 541 isolates were collected in clinical microbiology laboratory. Additionally, 112 isolates of several culture collections (ATCC, DSMZ) were analysed. All organism were grown overnight on the respective agar media. MALDI-TOF target plates were inoculated by applying a small amount of colony material directly onto the target plate in a thin film. Furthermore, an Ethanol/ Formic acid extraction procedure was compared to the direct method. The microbial film was overlaid with matrix solution and air dried. Measurement was performed in a MALDI-TOF spectrometer of the FLEX series (microflex LT, Bruker). Analysis of the generated peaks was done with the Biotyper 2.0 software (Bruker). Setup of VITEK2 was performed according to manufacturers instructions.
Discrepant results were resolved by sequencing of a 16S rDNA fragment
Results: The accuracy of the MALDI-TOF-system in identifying clinical bacterial isolates to species level was as follows: 94.9% for 271 Enterobacteriaceae tested, 78% for 65 nonfermenting Gram-negative rods (*Pseudomonas aeruginosa* showed 100% correct results), 92% for 115 staphylococci, 100% for 31 enterococci, 100% for 21 streptococci and 93% for 38 others. Of the 122 reference strains tested, 103 (92%) were correctly identified to species level.
Workflow time with the MALDI-TOF-system was calculated, including manual time, drying, import into the system, and time to result. The identification of one isolate with the direct smear method was available in 12 min and in 20 min with the extraction method. For one target plate (96 isolates) identifications were available in 1 h 45 min with the direct smear and in 3 h with the extraction method.
Conclusions: The MALDI-TOF system evaluated in this study proved to be a rapid and reliable method for the identification of the most relevant bacterial isolates in clinical routine laboratory.

P1965 **Influence of PCR parameters on the prevalence of Panton-Valentine leukocidin positivity in *Staphylococcus aureus* isolates**

Z.C. Karahan, I. Dolapci, A. Tekeli (Ankara, TR)

Objectives: Panton-Valentine leukocidin (PVL) is an important virulence determinant of *Staphylococcus aureus* strains. PCR amplification of the genes encoding PVL is the most widely used method for determining

PVL-positivity. In this study, we used two different primer sets and different annealing conditions for each set to demonstrate the effect of PCR components on PVL-PCR results.

Methods: 321 non-duplicate *S. aureus* strains and GRE-14 positive control strain were included in this study. PCR amplification of the PVL genes were performed by (luk-PV-1 and luk-PV-2) and (PVLup and PVLdn) primers by previously published methods. For the first primer pair PCR was performed at 55 ºC and 58 ºC, and for the second set of primer at 50ºC and 48 ºC with the same thermal cycler.

Results: The first primer pair gave 161 (50.16%) PVL-positive amplicons at 55 ºC and 12 (7.45%) at 58 ºC. The second primer set gave 0 (0%) and 5 (1.56%) PVL-positive amplicons at 50 ºC and 48 ºC respectively. The 161 PVL-positive amplicons were subjected to BspH1 restriction endonuclease analysis in order to confirm the accuracy of the amplified region, and 12 (7.45%) of the 161 isolates which were also found to be positive with the first set of primers at 58 ºC gave the expected restriction patterns of 180, 151 and 102 bp. Two of the 149 uncut amplicons were selected randomly and subjected to bi-directional automated sequence analysis with the same primers used for amplification, and the sequence obtained was compared with the sequences deposited to the GenBank. We found that 95% of this sequence was 99% identical to the genes encoding some conserved proteins of *Staphylococcus aureus*.

Conclusion: Our results show that primer selection and cycling conditions may lead to false-negative or false-positive results (due to cross-priming of the primers) in PVL-gene amplification. Restriction endonuclease or sequence analysis may be used to differentiate crossly-amplified sequences from true PVL-positive amplicons.

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P1966 Problems with real-time PCR for MRSA: why culture is still required

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Objectives: Real-time PCR processing can detect MRSA from nasal swabs. We describe the introduction of the IDI MRSA test into a clinical laboratory over a six-month period, run in parallel with traditional culture methods.

Methods: One biomedical scientist processed over 2000 nasal swabs using the IDI MRSA test. IDI MRSA positive-culture negative (false positive) lysates were retested using original and modified primers and the results crosschecked against additional MRSA culture findings from patients. IDI MRSA negative-culture positive (false negative) strains were referred for molecular analysis. Total costs of IDI MRSA and routine culture were calculated and compared.

Results: A valid IDI MRSA result was achieved for 75% of swabs tested. About 50% were IDI MRSA and culture negative, 10% were IDI MRSA and culture positive, 0.5% (nine) were IDI MRSA negative-culture positive and 18% were IDI MRSA positive-culture negative. Some 14% samples remained 'unresolved' by IDI MRSA, six of which were culture positive. Repeat testing with modified primers in combination with additional culture results showed that 316 of 362 (87%) 'false positives' were true positives. Seven of nine false negative strains contained mecA, and thirteen of fourteen meticillin-susceptible isolates gave false positive IDI MRSA results, depending upon which preparatory method was used. Sensitivity and specificity of the IDI MRSA method were 93.5% and 60.3%, respectively. Positive predictive value (PPV) was only 37.3%. We recalculated the PPV using additional microbiological data and found that it increased to 90%, with specificity increasing to 74%. Negative predictive value was 99.1%.

Conclusions: The IDI MRSA method was cost neutral compared with routine culture (£14.30 vs £14.80 per test) but results were obtained for only 75% of samples. Further and continued work is needed on the molecular components of this assay. We discuss the reasons for unresolved tests, invalid controls and misleading results. Culture and isolation of MRSA still remains the 'gold standard' where patients are concerned.

P1967 Molecular screening of respiratory specimens from paediatric cystic fibrosis patients to enhance detection of *Pseudomonas aeruginosa*

E. McCulloch, C. Lucas, C. Williams (Glasgow, UK)

Objectives: Cystic Fibrosis (CF) is an incurable, genetically inherited disease that affects some 1 in 3,000 individuals born in the UK. Chronic colonisation of the lungs with *Ps. aeruginosa* has a severe impact on respiratory function, which may be delayed by aggressive antibiotic therapy following detection of initial colonisation. The risk of acquiring *Ps. aeruginosa* increases with age, therefore particular emphasis is placed on the detection of the organism in respiratory secretions in younger patients. We have investigated whether a real time PCR method would be more sensitive than conventional microbial culture for the detection of *Ps. aeruginosa* in this setting.

Methods: In our real-time PCR assay, DNA is extracted using the EZ1 BioRobot (Qiagen) and a tissue extraction kit, then amplified and detected using an ABI Prism 7000 sequence detector and Taqman probes (Applied Biosystems). Each sample is performed in duplicate using a reaction with which amplifies a specific *Ps. aeruginosa* gene target allowing detection DNA of interest. The specimens were also cultured as per our standard laboratory procedure.

Results: Of the 373 samples tested (from 145 patients) 336 were negative by both culture and, 11 were positive by both methods but 25 (from 24 patients) were positive by PCR when culture was negative No false positive PCR results were detected from all culture negative swabs. The specificity of the real-time PCR method was confirmed by sequencing PCR products from positive specimens and matching to *Ps. aeruginosa* by BLAST search of the NCBI database.

Conclusion: The use of real-time PCR on cough swabs allowed a significant improvement over routine culture in the detection of *Ps. aeruginosa*. Given the benefits of early institution of antimicrobial therapy to eradicate carriage, this method has the potential to further enhance the survival of CF sufferers.

While PCR generated clinically useful information, genetic relatedness between strains first detected by PCR and strains which go on to establish colonisation, remains to be established. Whether *Ps. aeruginosa* can be spread from PCR positive/culture negative patients and what the effect earlier antibiotic treatment might be on generation of multi-resistant strains, are currently matters of speculation.

P1968 Evaluation of the prevalence of middle ear pathogens in the outer ear canal and nasopharyngeal cavity of a healthy population

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Objectives: Currently there are several studies investigating the role of *Alloiococcus otitidis* as a potential pathogen in otitis media with effusion. The objective of this study was to investigate the prevalence of the known middle ear pathogens in the outer ear canal and the nasopharyngeal cavity for a healthy population ($n = 70$).

Methods: For each of a group of 70 healthy students one swab each of the outer ear canal and of the nasopharyngeal cavity was collected. The swabs were sent to the laboratory where they were resuspended in 250 μl physiological water. After this homogenisation 200 μl was transferred to 800 μl EasyMag extraction buffer and DNA was extracted using the NucliSense EasyMag automated extractor. Species-specific PCR was carried out on all samples for *Alloiococcus otitidis*, *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Haemophilus influenzae* and *Pseudomonas aeruginosa*. For a subset of 10 outer ear samples, culture was carried out to investigate the possibility to detect *Alloiococcus otitidis* by culture. Culturing was done by incubating the samples on a blood agar plate (Tryptic Soy Agar + 5% sheep blood)

during 5 days at 37ºC, followed by subculturing the small, mostly gamma haemolytic colonies on Heart Infusion Agar plates (containing 5% sheep blood).

Results: Percentage of positive PCR results for the 70 patients are presented in Table 1.

Table 1. Species-specific PCR results

Detection	Outer ear canal swabs	Nasopharyngeal swabs
A. otitidis	80	3
H. influenzae	7	13
M. catarrhalis	0	34
S. pneumoniae	0	9
P. aeruginosa	14	19

For subgroup of 10 people the samples were also cultured. In this group PCR revealed 9 out of 10 positive for *A. otitidis* by PCR, and we were able to detect small colonies for 5 samples in this subgroup after long incubation periods. We were able to identify these colonies afterwards as *A. otitidis* by sequencing.

Conclusion: *Alloiococcus otitidis* was found to be present in 80% of the outer ear canal of a healthy study population. Detection can be carried out in a fast way by automated DNA-extraction on homogenised swab, followed by species-specific PCR. Culture is possible but requires long incubation, increasing the chance of overgrowth by other bacteria and decreasing sensitivity, compared to PCR.

M. catarrhalis and *S. pneumoniae* were found to be present in the nasopharynx with percentages of resp. 34 and 9%, but were not found once in the outer ear. *H. influenzae* and *P. aeruginosa* could be demonstrated to be present in both the nasopharynx and the outer ear in percentages ranging from 7 to 19%.

P1969 Automated DNA extraction with the easyMAG prior to molecular investigation of blood-borne bacteria and fungi with SeptiFast

M. Alvarez, S. Carlos, A. Peña, N. Chueca, M. Torres, J.A. Pérez-López, G. Piedrola, M.C. Maroto, F. García (Granada, ES)

Objective: to evaluate the performance of an automated extraction system for processing whole blood and sterile fluid samples prior to amplification and detection with the SeptiFast.

Methods: Whole blood K-EDTA anticoagulated samples were spiked with 300 and 30 UFC/ml of *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* or *Candida albicans*. DNA was extracted in parallel with the Nuclisens® easyMAGTM (BioMérieux) and with SeptiFast Lys Kit MGRADE and SeptiFast Prep Kit MGRADE (LightCycler® SeptiFast, Roche Diagnostics). The Nuclisens easyMAG Specific A ("off board") extraction protocol add 1 mL blood to the lysis buffer (guanidine thiocyanate). Amplification and detection were run in parallel after both extraction methods. 40 sterile fluids were tested with the automated extraction.

Results: EasyMag DNA extraction was able to detect 300 UFC/ml in: 1/1 for Gram-positive, 2/3 for Gram-negative; 30 UFC/ml in: 1/1 for Gram-positive, 2/4 for Gram-negative and 1/1 for *Candida albicans*. SeptiFast extraction could detect 300 UFC/ml in: 1/1 for Gram-positive, 4/4 for Gram-negative, 1/3 for *Candida albicans*; 30 UFC/ml in: 1/1 for Gram-positive, 1/3 for Gram-negative and 0/2 for *Candida albicans*. No contamination of the negative controls with the automated system were noted. 30% of sterile fluids were positive using the easyMAG extraction (*Streptococcus* spp., *Escherichia coli*, *Stenotrophomonas maltophilia*, CoNS, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*).

Conclusions: EasyMAG has proven to be a versatile, reliable and adequate method for the extraction of bacterial and fungal DNA prior to amplification with the SeptiFast system, resulting in an improvement of the workflow and hands on time (by two hours) of this novel molecular method for the diagnosis of bacteria.

We would like to aknowledge Nora Mariela Martinez (Biomerieux España) for technical assistance.

P1970 SeptiFast for the investigation of bacterial and fungal DNA in sterile fluids

M. Alvarez, J. Parra, A. Peña, S. Carlos, N. Chueca, M. Torres, J.A. Pérez-López, G. Piedrola, M.C. Maroto, F. García (Granada, ES)

Objective: to investigate the diagnostic performance of a novel molecular tests for the diagnosis of pathogenic bacteria and fungi (SeptiFast, Roche Diagnostics) in sterile fluids coming from joints, pleura and peritoneum, and to compare molecular findings with conventional culture microbiological methods.

Patients and Methods: 50 samples from 44 patients were included in the study. Samples corresponded to synovial fluid (n = 17), pleural fluid (n = 15), ascytic fluid (n = 9), peritoneal fluid (n = 3), and 6 samples were bone/tissue/prosthesis samples from prosthetic joint infection. All the samples were studied using the LightCycler® SeptiFast Test MGRADE (Roche Diagnostics), a multiplex real time PCR that in six hours investigates Gram-positive, Gram-negative, and species of *Aspergillus* and *Candida*; in parallel, standard microbiological procedures run in our laboratory were applied to all the samples.

Results: overall, 30% of the samples were positive using Septifast and 14% using conventional culture. Bacterial DNA from Coagulase Negative Staphilococci (n = 5), *Staphylococcus aureus* (n = 4), *Streptococcus* spp. (n = 2), *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, and *Escherichia coli*. Two of the *Staphylococcus aureus* detected with Septifast were also isolated by convencional methods. Culture positive/Septifast negative results were present in 5 samples (*Staphylococcus aureus*, *Peptostreptococcus anaerobius*, *Streptococcus viridans*, *Streptococcus agalactiae* and *Streptococcus pneumoniae*).

A subanalysis taking into account the type of sample investigated revealed that 66.6% of the joint infection related samples were positive by both methods (2 Septifast/Culture positive and 2 Septifast negative/Culture positive), 55.5% of the ascytic fluids (4 Septifast positive/culture negative and 1 Septifast negative/culture positive), 46.6% of the pleural fluids (5 Septifast positive/culture negative and 2 Septifast negative/culture positive). All synovial fluids were culture negative, and two (11.8%) were positive by Septifast.

Conclusion: Using Septifast, DNA from the panel of bacteria investigated could be detected in a large number of negative culture samples. However, the finding of samples with a positive conventional culture result and a negative Septifast test sets the rationale, if possible, for the use of both tests for the diagnostic microbiology of these samples.

P1971 A diagnostic algorithm for accurate identification of non-fermentative Gram-negative rods and epidemic strains of *Pseudomonas aeruginosa* from cystic fibrosis patients

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Objectives: Identification of non-fermentative Gram-negative bacteria using phenotypic tests such as biochemical profiling and chromogenic agar is difficult. Accurate identification is most important in individuals with cystic fibrosis (CF) as some species e.g. *P. aeruginosa* are associated with chronic respiratory infection whilst the role of others e.g. *A. xylosoxidans* is less well defined. Even amongst *P. aeruginosa* some strains such as the Liverpool Epidemic Strain (LES) are associated with heightened transmissibility and virulence. We developed a diagnostic algorhithm involving specific PCR and 16S rDNA sequencing for the accurate identification of CF isolates

Methods: Non-fermentative Gram-negative rods were isolated from CF sputum by standard techniques. Phenotypic identification of oxidase positive organisms was attempted by colonial morphology using *Pseudomonas* isolation agar and API 20 NE. PCR for *P. aeruginosa* specific genes (eta 396 Bp) and LES specific sequences (LES9 431 bp and PS21 400 bp) was applied to 597 consecutive isolates. Isolates

negative by *P. aeruginosa* specific PCR were identified by sequence analysis of the 16S rDNA gene.

Results: Phenotypic identification yielded *P. aeruginosa* (n = 446), *Burkholderia* spp. (n = 6), *S. maltophilia* (n = 5), *A. xylosoxidans* (n = 2) and R. pickettii (n = 1). 139 isolates could only be identified as 'non-fermenters'. 525 isolates were positive for *P. aeruginosa* specific PCR. Sequence analysis of the 16S rDNA gene of the remaining isolates yielded; *A. xylosoxidans* (n = 19), *Burkholderia cepacia* complex (n = 10), *P. aeruginosa* (n = 10), P. putida (n = 9), *S. maltophilia* (n = 9), *Chryseobacterium* spp. (n = 7) along with Agrobacterium, *Bacillus licheniformus*, *Bacillus simplex*, *Pseudomonas fulva*, *Proteus mirabilis*, *Neisseria elongata*, Enterobacteriaciae, Sphingomonas multivorum (all n = 1). The LES strain of *P. aeruginosa* was identified in 76 isolates from 21 patients.

Conclusions: Phenotypic identification of non-fermentative Gram-negative bacteria from CF patients is extremely unreliable. A combination of *P. aeruginosa* specific PCR with 16S sequence analysis of negative isolates is a useful approach for the identification and detection of classical and 'emerging' pathogens in CF.

P1972 Routine-based evaluation through a 3-year survey of the commercial Chlamylege assay for direct identification by multiplex PCR of *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, *Legionella pneumophila* and *Legionella* spp. in human respiratory infection

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Objectives: Chlamylege is a commercial assay developed by Argene Biosoft (Varilhes, France) for the detection and identification of *M. pneumoniae*, *C. pneumoniae* and *Legionella* spp. in respiratory samples, by using a multiplex PCR-hybridisation method. The present study evaluated the ability of this test to detect atypical pathogens in samples of patients presenting a respiratory infection, received on a routine basis in the Microbiology laboratory of a University Hospital through a 3-year period.

Methods: A total of 2169 respiratory specimens including 1218 (56.1%) samples from paediatric origin were tested once-a-week through a 35-month period; they consisted of nasopharyngeal aspirates, tracheal secretions and bronchoalveolar lavages. After a preliminary treatment of the sample by proteinase K, the Chlamylege assay was performed on DNA samples extracted manually with silica-based columns in 2005, with a Kingfisher apparatus (Thermolabsystems) in 2006, and with an Easymag instrument (Biomérieux) in 2007. In combination with the latter extraction method, the result was available within two days for runs of 20 to 25 samples.

Results: The routine use of the Chlamylege assay led to the detection of the genome of at least one atypical respiratory pathogen in 189 of the 2169 samples (8.7%), including 162 *M. pneumoniae*, 18 *C. pneumoniae*, 10 *Legionella pneumophila* and 3 *Legionella* spp. signals, respectively. Only 6 samples were shown to contain PCR inhibitors. All but 5 *M. pneumoniae* infections were diagnosed in infants and young adults. A retrospective analysis of the medical files of patients with samples found positive with the Chlamylege assay revealed a high prevalence of multiple infections since *M. pneumoniae* was associated to a high amount of another bacterial species in 30% of the cases and with a viral infection in 12% of them. Interestingly, a familial outbreak involving both *M. pneumoniae* and *C. pneumoniae* infection was documented.

Conclusion: The use on a routine basis of a sensitive molecular assay to detect simultaneously different atypical pathogens in respiratory samples is a convenient strategy to detect and identify rapidly fastidious-growing bacteria and to treat efficiently infections due to β-lactams resistant agents. In addition, this study underlines that the place of *M. pneumoniae* in common respiratory infections is largely underestimated when molecular tests are not available.

P1973 Molecular diagnostic of sepsis: episodes vs. cases

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Objectives: To evaluate the interpretation of the data obtained after utilising a multiplex PCR assay that identifies relevant microorganisms in septic patients. Data analysis of episodes (samples taken at same time) vs. cases (serial samples in same patient) were compared.

Methods: Sensitivity, sensibility, positive and negative predictive values were determined in a group of 53 patients from our post surgical/ high-complexity ICU with systemic inflammatory response syndrome (SIRS) and high clinical risk factors for bloodstream infection. Agreement was measured by kappa test.

Confirmation by microbiological identification of a pathogen in culture of blood or other locations (conventional microbiology), was used as a standard for determining if patients were infected or non-infected. The evaluation was retrospective and observational. In all cases the patients were covered by aggressive empiric therapy and gave their informed consent.

PCR results were obtained using multiplex Septifast test (ROCHE), blood cultures were done with BactAlert and conventional identification was performed with VITEK 2 (Biomerieux).

Results: 366 Septifast determinations were obtained for the 53 patients included in the study. We paired blood cultures and Septifast determinations in 294 episodes, conventional microbiology and Septifast in 302 episodes, and conventional microbiology plus blood culture vs. Septifast in 294 episodes. All 53 patients were serially analysed on days 0, 1, 2, 3, 7, 14 and 28 after ICU admittance the results were analysed using Septifast as a standard for determining disease or non-disease status. These results were then matched to see if coincidence existed with the results from blood and peripheral compartments obtained consecutively over the time span of a week using conventional phenotypic methods. Results are in Figure 1.

ANALYZING EPISODES (all samples taken at same time) vs. CASES (series of 8 consecutive "episodes")

Blood culture or/and conventional microbiology defines disease condition.
SEPTIFAST™ defines disease condition (early detection).

- results include contaminants.

Guidelines for Strength of Agreement Indicated with κ Values

κ Value	Strength of Agreement beyond Chance
<0	Poor
0–0.20	Slight
0.21–0.40	Fair
0.41–0.60	Moderate
0.61–0.80	Substantial
0.81–1.00	Almost perfect

Note.—Data are from Landis and Koch (8).

	Episodes			Cases		
	Blood culture vs. SeptiFast	Conventional Microbiology vs Septifast	Blood Culture plus Conventional Microbiology vs Septifast	Blood culture vs. SeptiFast	Conventional Microbiology vs Septifast	Blood Culture plus Conventional Microbiology vs Septifast
Sensitivity	26.13 (17-36)	19.87 (14-26)	**27.41 (21-34)**	52.17 (30-73)	54.16 (32-74)	**75 (53-90)**
Specificity	84.68 (79-89)	84.82 (76-90)	**90.16 (79-96)**	76.66 (57-90)	46.66 (28-65)	**86.66 (69-96)**
Positive predictive value	41.81 (28-55)	66 (51-78)	**89.47 (78-96)**	63.15 (38-83)	44.82 (26-64)	**81.81 (59-94)**
Negative predictive value	73.14 (67-78)	41.66 (35-48)	**28.94 (22-35)**	67.64 (49-82)	56 (34-75)	**81.25 (63-92)**
Prevalence	29.62	59.71	**75.3**	43.39	44.44	**44.44**
Likelihood ratio Positive test	1.7 (1.06-2.74)	1.3 (0.76-2.23)	**2.78 (1.25-6.17)**	2.23 (1.04-4.76)	1.01 (0.61-1.67)	**5.62 (2.19-14.41)**
Likelihood ratio Negative test	0.872 (0.76- 1)	0.944 (0.847-1.05)	**0.804 (0.713-0.9)**	0.623 (0.389-0.99)	0.982 (0.55-1.75)	**0.288 (0.142-0.58)**
Proportion agreement (8)	0.67 (slight)	0.46 (slight)	**0.42 (slight)**	0.64 (fair)	0.53 (slight)	**0.81 (substantial)**
Bias Index	-0.11	0.41	**0.52**	-0.09	0.05	**0.03**
Prevalence Index	-0.51	-0.22	**-0.01**	-0.2	-0.05	**-0.14**
kappa	**0.121**	**0.04**	**0.102**	**0.272**	**0.074**	**0.621**

- (*) Note: (95% Confidence interval calculated with binomial expansion)

- (8) Landis RJ , Koch GG. The measurement of observer agreement for categorical data. Biometrics 1977; 33:159-174.

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Figure 1.

Conclusion: Septifast test detects the special condition of DNAhemia. This condition is independent of the viability needed for microbial growth in cultures. The sensitivity (75%) and specificity (86.66%), positive (81.81%) and negative (81.25%) predictive values and kappa (0.621) all indicate that the molecular test results are substantially (0.81) related to patient infection by microorganisms (located in blood or other compartments). As these patients tend to be refractory to conventional bacteriological diagnostic approaches, molecular methods may eventually prove to be a valuable criteria for early adequate antimicrobial treatment.

P1974 Multiplex PCR for the detection of bacterial infections in human faeces

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Objectives: Diarrhoeal diseases are responsible for thousands of paediatric deaths per day in developing regions, and the associated co-morbidity impacts on childhood development, nutritional status and cognitive function. The aim of this project is to develop molecular diagnostic tools for multiple enteric pathogens in order to (1) improve disease surveillance and (2) gain more insight in the possible contribution of *Helicobacter pylori* in the epidemiology of diarrhoeal diseases in developing countries.

Methods: We developed multiplex real-time (rt-)PCR assays for detection of *H. pylori*, *Salmonella* spp., *Shigella* spp., and *Campylobacter jejuni*. Specificity of the primers was investigated by testing these on a large panel of bacterial samples. Sensitivity was determined by performing rt-PCR reactions on dilution series of the specific bacteria, both in medium and in spiked human faeces. We isolated bacterial targets from spiked human faecal samples using four isolation methods; (1) MagNA-Pure LC isolation after pre-treatment with S.T.A.R. buffer (Roche), (2) EZ1 biorobot isolation using the EZ1 DNA Tissue Kit (Qiagen), (3) QiaCube isolation using the QiaAmp DNA Stool Mini Kit (Qiagen) or (4) manual isolation using the QiaAmp DNA Stool Mini Kit. Clinical validation on stool samples is ongoing.

Results: All primer sets are highly specific and did not yield amplification products for any of the bacteria other than the specific targets, using up to 10^6 CFU/PCR reaction as input material. Primer sets allowed amplification of 1 CFU/PCR reaction of a bacterial solution in medium. Amplification of targets isolated from spiked faecal samples showed that a bacterial concentration of 1^3–1^4 CFU/ml faeces was the lowest detectable, depending on the target and the isolation method. This corresponds to 1–10 CFU input per PCR reaction. Isolation using the Qiagen EZ1 biorobot system proved to be the method of choice for isolation of bacterial targets from faeces, yielding higher sensitivities and showing less PCR inhibition than the other methods tested.

Conclusion: We have developed a multiplex rt-PCR assay for the detection of *H. pylori*, *Salmonella* spp., *Shigella* spp., and *C. jejuni*. in faecal samples that, combined with the Qiagen EZ1 biorobot isolation system, allows detection of 1^3–1^4 CFU bacteria per ml faeces.

P1975 Comprehensive study comparing a PCR based monitoring method, solid phase cytometry and standard routine methods from an artificially controlled water sample of *Legionella pneumophila* Paris strain: the importance of detecting VBNC

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Objectives: *Legionella pneumophila* is the causative agent of Legionnaires' disease. It is an ubiquitous bacterium of aqueous environments, which requires amoebas as an intracellular replicative niche. Real-time PCR based methods have been developped for rapid detection of *Legionella* DNA in water samples. *L. pneumophila* have been reported to enter a viable but non cultivable (VBNC) state, which can be detected by PCR. In addition of VBNC and cultivable *L. pneumophila*, PCR could also detect the presence of dead bacteria.

Methods: In order to understand the significance of a positive PCR result, controlled artificially contaminated water samples with and without chlorination treatment were enumerated for *L. pneumophila* by conventional growth methods, real time PCR, viability labeling and immuno-detection assay using solid phase cytometry.

Results: Here, we show that while a clear correlation was observed between theses techniques for the detection of *L. pneumophila* in non-treated water, important discrepancies were seen for treated-water samples. After 24 h with 0.5 and 1 ppm of chlorine treatment, the culture was negative, whereas concentrations given by PCR and immunodetection were still about 106 UG/mL, and the metabolically active bacteria measured by TVC detected 105 and 102bacteria/mL, respectiveley. Thus, positive PCR signal includes the presence of bacteria that were viable (with a positive viability signal) and not cultivable (no growth on plate), that is VBNC. To confirm this result, VBNC of *L. pneumophila* Paris strain, which have been obtained using 0.5 ppm of chlorine for 24 h and were detected by PCR and immunodetection but not on plates, recovered their culturability after 5 days of co-culture with *A. polyphaga*. Therefore, some of the TVC positive bacteria remained infective.

Conclusions: Taken together, these data show that a PCR positive signal includes VBNC and dead bacteria in addition of cuturable bacteria. PCR represent a very quick, cost effective and reliable tool to detect bacteria in treated waters that can be potentially associated with a health risk.

P1976 16S rDNA sequence analysis can help in revealing the aetiologic agent of culture-negative infective endocarditis

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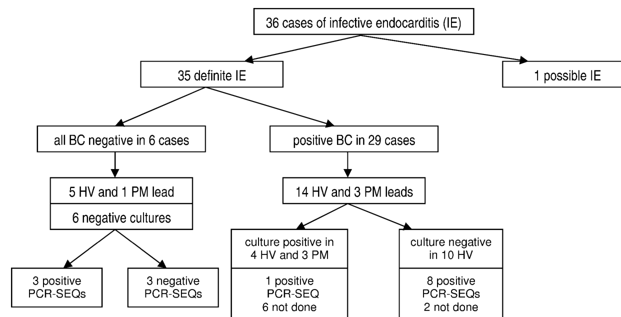
Objectives: The diagnosis of infective endocarditis (IE) is based upon initial clinical suspicion, microbiological data and echocardiographic findings. Despite the use of appropriate laboratory techniques, classic microbiological diagnostics (blood cultures or serology) do not always provide an aetiological diagnosis. In order to increase the aetiological diagnosis of IE, the use of PCR followed by sequence analysis (PCR-SEQ) was assessed.

Methods: From June 2006 to November 2007, 36 IE cases were reported to the clinical laboratory, according to the Duke criteria (35 cases definite, 1 case possible). From all patients at least 2 sets of blood cultures (BC) were available. From 24 patients surgical samples [heart valves (HV n= 20) and pacemaker leads (PM, n = 4)] were received. When no aetiologic agent was identified, samples were analysed by nested PCR with primers on 16S-rDNA followed by sequencing and BLAST-analysis.

Results: In 29 of the 35 definite IE cases, an aetiologic agent was proved by positive BC. From 17 of this 29 cases, surgical samples were received and 7 were culture positive. In the 10 other samples, which remained culture negative, PCR-SEQ (performed on 8 samples) revealed an aetiological agent in 8 samples. Germ identification with PCR-SEQ was in all cases identical with the culture results.

In 6 cases all cultures (BC and cultures of the 5 HV and 1 PM lead) remained negative. However, in 3 out of 6 an unambiguous sequence was found by PCR-SEQ pointing to the causative infective agent. Results are shown in fig 1.



PCR-SEQ demonstrated the presence of streptococci (n = 6), including enterococci, *Propionibacterium acnes* (n = 1), staphylococci (n = 2), *Listeria monocytogenes* (n = 1), *Neisseria elongata* (n = 1) and *Cardiobacterium hominis* (n = 1). In the 3 patients from whom all cultures remained negative but PCR-SEQ was positive, a history of previous antibiotic treatment was available.

Conclusion: The application of molecular diagnostics is useful in patients with IE in whom no causative agent can be found. Cultures are often negative because of the administration of antibiotics prior to sample collection. In all tested BC positive cases, PCR-SEQ confirmed

the aetiologic agent. In 3 out of 6 culture negative cases, PCR-SEQ could reveal the aetiologic agent.

P1977 Evaluation of a modified octaplex PCR assay for the detection of major diarrhoeagenic *Escherichia coli*

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Objectives: To evaluate a modified PCR assay for identification of diarrhoeagenic *Escherichia coli* (DEC) and *Shigella* spp. by simultaneous and specific detection of seven virulence genes and a 16S rDNA internal PCR control. The modified PCR assay is based in part on previously published primer sequences and has recently been multiplexed by Brandal et al.

Methods: PCR target genes were: verocytotoxins 1 (vtx1) and 2 (vtx2), characteristic of verocytotoxin-producing *E. coli* (VTEC); intimin (eae), found in enteropathogenic *E. coli* (EPEC), attaching and effacing *E. coli* (A/EEC) and VTEC; heat-stable enterotoxin (estAh) and heat-labile enterotoxin (eltB), characteristic of enterotoxigenic *E. coli* (ETEC); aggR for enteroaggregative *E. coli* (EAEC); and invasive plasmid antigen (ipaH), characteristic of enteroinvasive *E. coli* (EIEC) and *Shigella* spp. The assay performance was tested with a strain collection that included four non-pathogenic *E. coli* strains, five non-*E. coli* species, eight *Shigella* spp. and 71 different DEC strains, representing a variety of different serotypes and virulence profiles: VTEC (n = 20), EPEC (n = 33), A/EEC (n = 8), ETEC (n = 5), EAEC (n = 3), EIEC (n = 2). In addition, 160 clinical faecal samples were tested.

Results: The method allowed the simultaneous identification of all eight genes in one reaction. When applied to pure cultures from the strain collection, virulence genes were identified in 68/71 (96%) of the different DEC strains, in all *Shigella* spp. and no virulence genes were detected with the four non-pathogenic *E. coli* strains and five non-*E. coli* species. The three negative DEC strains (two EPEC and one A/EEC) were positive on repeated testing and suggest a procedure error at initial testing. Among the clinical specimens, 16/160 (10%) were positive for one or more virulence gene: EPEC (n = 1), A/EEC (n = 8), ETEC (n = 2), EAEC (n = 4) and EIEC (n = 1).

Conclusion: The modified octaplex PCR assay provides a simple, rapid and inexpensive diagnostic procedure for simultaneous detection of major diarrhoeagenic *Escherichia coli* and *Shigella* spp. in the routine diagnostic laboratory.

P1978 Evaluation of PCR for identifying *Brucella* species isolated from blood from patients living in a non-endemic area

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Objectives: Brucellosis is rare in Denmark. Primary laboratories are not prepared for handling these BSL class 3 organisms, and there is a considerable risk of laboratory staff being exposed to the bacterium. Traditionally, isolates of *Brucella* species has been referred to our national reference laboratory and final identification has been made by a combination of culture and agglutination by antisera. As speed, safety and reliability are crucial factors in identification of *Brucella* species we evaluated these factors using PCR as an alternative.

Methods: Seventeen isolates from blood were analysed by PCR using primer sets for *B. melitensis*, *B. abortus*, and *B. suis*, respectively. The three PCR analyses were run in parallel and had PCR products of different size. Eight isolates previously identified by culture and agglutination by antiserum, were re-examined by PCR, and nine consecutively referred isolates were identified by PCR only. Two of the previously identified isolates had been reported as *B. melitensis*, four as *B. abortus*, and two as *Brucella* species.

The PCR test was completed in less than one working day, whereas cultivation and agglutination required at least overnight incubation.

Results: All isolates were postive for *B. melitensis* by PCR. One isolate was repeatedly positive in PCR for *B. abortus* in addition to *B. melitensis*, however the PCR product in the *B. abortus* assay did not show the expected size. The DNA sequence of the PCR product from the *B. abortus* PCR did not allow discrimination between the species. None of the isolates were identified as *B. abortus* or *B. suis* by PCR.

Conclusions:

1. PCR is faster than culture and agglutination. A definite conclusion of whether a suspected isolate is a clinical relevant *Brucella* species can be obtained within a few hours.
2. PCR is less hazardous than culture and agglutination. Isolates referred to the reference laboratory are immediately inactivated in DNA extraction buffer, allowing all subsequent procedures to be carried out in BSL class 2 facilities. The suspected isolate may even be inactivated, e.g. by heating, before shipment. In contrast to live *Brucella* bacteria, inactivated material may be send by ordinary means of transportation.
3. PCR results in definite species identification and there are no difficulties in interpretation of agglutination obtained by more or less cross-reacting antisera.

PCR for *Brucella* species is a fast, safe, and reliable method for identification of clinical isolates.

P1979 Real-time PCR detection of enterovirulent *Escherichia coli*

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Enterovirulent *Escherichia coli* are a common cause of morbidity throughout the word. The major categories of pathogenic *E. coli* include enterohaemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC) and enteroinvasive *E. coli* (EIEC). Targeting specific genetic elements i.e. vt1 and vt2 (EHEC), eaeA (EHEC/EPEC), bfpA (EPEC), estA and eltB (ETEC) and ial (EIEC/*Shigella*) we have developed a TaqMan-based real time PCR system to detect these pathogens from primary streaks of stool cultures. Using a common PCR protocol for all the selected virulence genes we were able to detect between 1 and 10 genomic equivalents for all selected targets (except the variant vt2d which was detected at 100 genomic equivalents). The specificity of the reactions was 100% when tested against 32 different bacterial strains commonly present in clinical samples. To validate the protocol both control strains, n = 212 (32 EHEC, 36 EPEC, 97 ETEC and 47 EIEC/*Shigella*) and primary streaks of stool cultures, n = 139 (62 EHEC, 17 EPEC, 22 ETEC and 38 EIEC/*Shigella*) were tested and compared to a well established diagnostic gel based PCR protocol. In total, eleven discrepancies were observed; vt1/vt2 (n = 5), estA/eltB (n = 5) and bfpA (n = 1) which all could be explained by the higher sensitivity of the real time protocol. Furthermore, after using the system in routine diagnostics for up to one year the variation of the reactions were around 2 percent (CV = 1.3–2.1%). We were also able to correlate the frequency of isolated strains to the amount of target DNA present in the initial samples. On this basis we have developed a cut off value which likely corresponds to the lowest DNA content representing presence of viable pathogens. This raise the question of how to deal with weakly positive samples from a system based on nucleic acid detection. In conclusion, we have developed a rapid and robust TaqMan based assay for routine diagnostics of enterovirulent *Escherichia coli*. Moreover, the method opens up for studies on clinical relevance in correlation to low DNA content in clinical samples.

P1980 Detection of *Mycoplasma hominis* in amniotic fluid of pregnant women by real-time PCR

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Objectives: Maternal infection has long been known to play a major role in perinatal morbidity and mortality and suspected to be a cause of spontaneous abortion. Intrauterine infection may be largely responsible for preterm delivery. The most common microorganism involved in intrauterine infections is *Mycoplasma hominis*. *Mycoplasma hominis* is commonly isolated microorganism from the female genital tract. It has been linked to pelvic inflammatory diseases such as salpingitis,

endometritis, bacterial vaginosis and infertility. The transmition from the mother to the developing foetus can be in utero or at delivery.
The relationship between detection of *Mycoplasma hominis* in mid-trimester amniotic fluid and subsequent pregnancy outcome was investigated. Data collected included indication for amniocentesis, gestational age at amniocentesis, gestational age at delivery, pregnancy outcome and low birth weight of infants.
Method: The amniotic fluids of 202 women who underwent a transabdominal amniocentesis at weeks 15–22 of pregnancy were tested for *Mycoplasma hominis* by real-time PCR.
Results: *Mycoplasma hominis* were identified in 51 (25.2%) samples of the amniotic fluid. A total of two (28.6%) infants, positive for *Mycoplasma hominis*, had the birth weight below 2500g. Ten of the women had preterm delivery and one of them (10%) was positive for *Mycoplasma hominis*.
Conclusion: In this study has not been found statistical correlation between *Mycoplasma hominis* and low birth weight and preterm delivery. Despite the detection of *Mycoplasma hominis* in samples of pregnant women should be considered. Real-time PCR is suitable for diagnostic of this microorganism in samples of amniotic fluid. This work was supported by MSM 0021627502.

P1981 Intrapartum screening of group B *Streptococcus* using GeneXpert real-time PCR versus late antenatal screening by culture

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Objective: Actual prevention of neonatal early onset group B streptococcal (GBS) disease involves giving intrapartum antibiotics to women. It takes in account either their antenatal culture colonisation status or for those women with unknown status, the presence of risk factors. The aim of this study was to evaluate the accuracy of GeneXpert real-time PCR, for the detection of GBS carriers at the onset of labor, and to compare it to the current 35 to 37 weeks screening by culture.
Methods: Over a six-month period 630 women were prospectively enrolled. According to ANAES recommendations in France, specimens were collected at 35 to 35 weeks by swabbing the lower third of the vagina, and cultured by direct plating on sheep blood agar.
At time of presentation for delivery, the vaginal swab was repeated using a double Copan swab. Of these, one swab was processed by culture, and the other one by GenExpert real-time PCR which integrates the all processing (extraction-amplification-detection) in 70 minutes.
Results: Of enrolled women 89% were eligible for analysis. The GBS colonisation rate when using culture, was 16.1% intrapartum versus 13.16% at 35 to 37 weeks. Compared with intrapartum culture, the sensitivity of late antenatal culture for identifying colonisation status at delivery was 54.1% and the positive predictive value 66.25%. PCR results were not available for 11% of the samples: 5% due to PCR inhibition, 3% due to significant presence of mucus and 3% due to manipulation errors. Compared with intrapartum culture, the GeneXpert real-time PCR test had a sensitivity of 97.9%, a specificity of 99.7%, a positive predictive value of 98.9% and a negative predictive value of 99.6%. The molecular test was superior to antenatal culture: sensitivity 97.9% versus 54.1%.
Interestingly 9% of negative screened women at 35 to 37 weeks became positive on intrapartum, and 34% of positive screened women became negative. Of screened positive women on time of labor, 47% were not detected by antenatal culture.
Conclusion: The real-time PCR on GenExpert is a rapid test, highly sensitive and specific to detect GBS colonised women at time of labor. This molecular test is extremely easy to use as a laboratory routine test. The results could be available 24H a day for antibiotics prescription to be given before delivery.

P1982 International external quality assessment programme for the molecular detection of *Legionella pneumophila* in respiratory samples: results from 2006 and 2007

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Objectives: To assess the diagnostic proficiency of laboratories performing nucleic acid amplification technologies for the detection of *Legionella pneumophila* in clinical specimens.
Methods: The 2006 EQA panel consisted of 12 bronchoalveolar lavage (BAL) or physiological saline solution samples, including eight samples spiked with *L. pneumophila* sg 1 or sg 6, two samples spiked with *Legionella micdadei* and two negative samples. The 2007 EQA panel consisted of 10 BAL or transport medium (Dulbecco's modified Eagle's medium and 10% foetal calf serum) samples including seven samples spiked with *L. pneumophila* sg1 or sg6, one with *L. micdadei* and two negatives. A scoring system was applied to compare performance: two points for a correct result and zero points for an incorrect result. A maximum score of 24 points was attainable in 2006 and 20 points in 2007.
Results: In 2006 a total of 58 datasets were returned from 51 respondents in 16 countries. The overall mean performance score was 17/24. Three percent (2/58), 14% (8/58) and 84% (49/58) of datasets exceeded 90%, 80% and 60% of the maximum score (24 points). There were 14 (12%) false positives reported for the two samples containing *L. micdadei*. The rate of false positives on the two true negative samples was 3% (3/116). In 2007, 65 datasets were submitted by 60 respondents in 21 countries. The overall mean performance score was 16/20. Twenty percent (13/65), 52% (34/65) and 89% (58/65) of datasets exceeded 90%, 80% and 60% of the maximum score (20 points). There were 19 (29%) false positives reported for the sample containing *L. micdadei*. The rate of false positives on the two true negative samples was 4% (5/130). A performance comparison between BAL and transport medium suggested that sample matrix may have influenced the results.
Conclusion: The overall performance improved from 2006 to 2007. However the percentage of false positives on true negative and specificity samples (*L. micdadei*) indicated that improvements are still needed in the detection of *L. pneumophila* by molecular methods.

P1983 Comparison of three methods: culture, real-time PCR and fluorescent in situ hybridisation in detecting of *Helicobacter pylori*

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Objectives: *H. pylori* is one of the most common bacterial pathogens in humans. It causes chronic gastritis, peptic ulcer disease and it is a major risk factor for gastric cancer. Detection of this pathogen with accurate methods is very important.
Aim: to compare *H. pylori* detection in gastric biopsy specimens by culture, Real-Time PCR and FISH.
Methods: Biopsy specimens of 148 patients, with dyspeptic complaints were examined. Culture was performed on Brain Heart Infusion agar, for Real-Time PCR examination, chromosomal DNA was extracted by Cetryl-trimethl-ammonium bromide method and the assay was performed on Light Cycler (Roche Diagnostics, Germany). Melting peaks of 650C (± 2.00C) showed the detection of *H. pylori* in the sample. For FISH examination formalin-fixed paraffin-embedded gastric biopsies were sectioned. The sections were hybridised using the commercially available test system seaFast *H. pylori* Combi-Kit (Theranostics International, Netherlands) according to the manufacturer's instructions. Four different sections were examined to give a negative result.
Results: Among 148 samples 70 were positive by culture, 118 were positive by PCR and 124 were positive by FISH. Among the 124 samples determined positive by FISH; 98 were determined positive in the first section, 17 were determined positive in the second section and 9 were

determined positive in the third section. Culture, PCR and FISH results were all positive for 63 samples and negative for 24 samples. 54 samples were culture negative, but PCR and FISH positive. 7 samples were PCR negative, but culture and FISH positive.
Conclusion: At least two tests are essential for *H. pylori* determination. Culture, because of contamination and the difficulties of culturing of *H. pylori*, has false negative results. However culturing must be performed, hence antimicrobial susceptibility testing is essential. Among the three methods Real-Time PCR has more capability to determine the positive results in the first sample, therefore it is easier to perform in routine laboratory practice.

P1984 Detection of bacterial pneumonia by quantitative PCR, with special reference to *Streptococcus pneumoniae* and *Haemophilus influenzae*

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Objectives: To develop sensitive and specific quantitative multiplex real-time PCR for detection of *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Neisseria meningitidis*.
Methods: A multiplex quantitative real-time PCR assay was developed for detection of *S. pneumoniae* (9802 gene fragment), *H. influenzae* (P6 gene) and *N. meningitidis* (ctrA gene). The analytical sensitivity of the assay was determined by serial dilutions of target DNAs. The method was evaluated on bronchoalveolar lavage from 156 immunocompetent adult patients with lower respiratory tract infection (LRTI) and 44 adult controls who underwent bronchoscopy for suspected malignancy. The PCR results were compared with the results of culture.
Results: The analytical sensitivity was 10–60 copies/reaction for *S. pneumoniae* and 3–10 copies/reaction for *H. influenzae*. The detection capacity was not affected by using a combined mixture of reagents and a combined DNA standard (*S. pneumoniae*/*H. influenzae*) in single tubes. By culture, *S. pneumoniae* and *H. influenzae* were aetiological agents in 21 and 33 of the LTRI patients, respectively.
These pathogens were identified by real time PCR in 45 and 67 of the cases, respectively, yielding sensitivities of 76% for *S. pneumoniae* and 90% for *H. influenzae*, and specificities of 78% for *S. pneumoniae* and 68% for *H. influenzae*. A cutoff for clinically significant positivity (10^4 DNA copies/ml for *S. pneumoniae* and 5×10^4 DNA copies/ml for *H. influenzae*) yielded sensitivities of 71% and 78%, and specificities of 85% and 86% for *S. pneumoniae* and *H. influenzae*, respectively. Of the 103 patients who had taken antibiotics prior to sampling, *S. pneumoniae* and *H. influenzae* were identified by culture in 7% and 17% of the cases, respectively, and by the PCR in 28% and 52% of the cases, respectively. Among the 44 controls, *S. pneumoniae* and *H. influenzae* were identified by culture in 3 and 5 cases, respectively, by PCR in 14 and 17 cases, respectively, and by PCR with the defined cutoff limits in 5 and 9 cases, respectively.
N. meningitidis was detected in 7 LRTI patients which indicated that the multiplex assay may be useful in the diagnosis of meningitis.
Conclusions: The multiplex format of the assay facilitates diagnosis of *S. pneumoniae* and *H. influenzae* and the assay enable detection after antibiotic treatment has been installed. Quantification increases the specificity of aetiology for pneumonia.

P1985 A new PCR assay for the diversity analysis of streptococcal populations in clinical samples

T. Mas-de-Xaxars, L.J. Garcia-Gil (Girona, ES)

Objectives: Streptococci are a heterogeneous group that includes more than 30 different species, most of them normal inhabitants of the human flora, although some constitute important human pathogens. No molecular tools to study the diversity of the genus *Streptococcus* in clinical samples have been developed, to date.
Methods: To study the diversity of this genus by the PCR-DGGE approach, we have developed a new set of primers consisting of a genus-specific forward primer targeting 16S rRNA (1043f) combined with the universal eubacterial primer 1492r. The ca. 450pb PCR product encloses three (V7, V8 and V9) hypervariable regions of the small subunit ribosomal gene and is therefore suitable to determine the specific diversity by denaturing gradient gel electrophoresis (DGGE).
Results: The specificity of the PCR assay was verified using 45 different bacterial species, including 16 different *Streptococcus* species. Positive PCR results were obtained in all *Streptococcus* species plus *Enterococcus faecalis*, whereas PCR was negative in the other specimens. The amplification limit was 900 genome equivalents per reaction (95% probability).
The new oligonucleotide set was tested on clinical (colon biopsies, bronco-alveolar lavage, and hip/knee prosthetic infections) samples. Up to 60% of the sequences retrieved from samples that resulted positive for streptococcal populations were highly similar to cultured streptococci. In turn, 40% of the sequences corresponded to yet uncultured streptococci, and some of them corresponded to new *Streptococcus* phylotypes. Furthermore, the new molecular tool was compared with isolation methods using colon biopsies samples. Of all streptococci identified, 70% were obtained by conventional culturing methods while 60% were derived from PCR-DGGE technique. Both methods shared 30% of total streptococci. The streptococci found only with molecular methods (30%) represent an abundant fraction and therefore are of potential clinical interest.
Conclusions: This new *Streptococcus*-oriented analytical tool will to provide a deeper insight into the specific composition of streptococcal populations, particular those from infected tissues and can be useful to identify new participants in polymicrobial infections.

P1986 Evaluation of PCR in bronchoalveolar lavage specimens for diagnosis of pneumococcal pneumonia

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Objectives: Due to the limitations of conventional methods, PCR has been extensively evaluated as an attractive diagnostic tool for pneumococcal pneumonia. To date most investigators focused on blood and sputum specimens with blood specimens showing wide range of sensitivities and sputum specimens showing difficulties in determining whether PCR-positive findings represent colonisation or infection, at least when conventional and not quantitative PCR methods are used. Bronchoalveolar lavage (BAL) is invasive specimen from lower respiratory tract with minimal risk of contamination by the oropharyngeal flora. Therefore the aim of this study was to evaluate PCR performed on BAL specimens for diagnosis of pneumococcal pneumonia.
Methods: The study included 80 adults with clinical and radiographic evidence of community-acquired pneumonia (CAP). Sputum, BAL (when indicated by physicians) and blood specimens were submitted for culture and urine specimens for urinary pneumococcal antigen test. PCR that targets the pneumolysin gene was performed on serum and BAL specimens.
Results: *Streptococcus pneumoniae* was detected in 25 of 80 (31.25%) patients. Urinary antigen test was positive in 16 of 76 (21.05%), sputum cultures in 8 of 64 (12.5%) and BAL cultures in 1 of 19 (5.26%) patients. There were no positive blood cultures. PCR results in 77 serum specimens were negative. BAL specimens were PCR-positive in 3 of 19 (15.79%) patients who underwent fibreoptic bronchoscopy. In two of those patients *S. pneumoniae* was detected only by BAL PCR. All 3 patients with positive BAL PCR were previously treated with antimicrobial agents.
Conclusion: According to our results in serum specimens, we agree with those investigators who claim that PCR performed on blood samples adds little to existing diagnostic tests for pneumococcal pneumonia. We conclude that BAL is the most promising specimen in diagnosis of pneumococcal pneumonia by PCR method, especially in patients previously treated with antimicrobial agents. Because BAL is invasive

procedure, BAL PCR would be ideal for patients with slowly resolving pneumonias when decision about further antimicrobial treatment should be made. However, evaluation on larger number of BAL specimens is needed.

Molecular diagnosis of miscellaneous micro-organisms

P1987 Identification of species in the *Acinetobacter calcoaceticus–A. baumannii* complex isolated from blood cultures by an oligonucleotide assay

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Objectives: *Acinetobacter calcoaceticus* (genomic species 1), *A. baumannii* (genomic species 2), and unnamed genomic species 3 and 13TU are phenotypically similar and were referred to as a group, the *A. calcoaceticus–A. baumannii* (ACB) complex. Members of the complex are the most common acinetobacters among clinical isolates. The aim of this study was to construct an oligonucleotide array with probes based on the ITS sequence to differentiate different species in the ACB complex and to determine the species distribution of 291 blood isolates of the complex.
Methods: Four oligonucleotide probes (18- to 30-mers), based on the intergenic spacer (ITS) sequences, were designed to identify the four genomic species in the ACB complex. An *Acinetobacter*-specific and an ACB complex-specific probe were also designed. In addition, a positive control probe was designed from a highly conserved region in the 16S rRNA gene. The array (4 by 4 mm) contained 12 dots (4 by 3 dots), including one *Acinetobacter*-specific probe, one ACB complex-specific probe, four probes for identification of each of the four genomic species in the ACB complex, one positive control probe, two negative control dots (tracking dye only), and three dots of the position marker probe. One pair of bacteria-specific universal primers 2F and 10R was used to amplify the ITS regions, with each of the two primers labeled with a digoxigenin molecule at its 5’ end. The amplified PCR products were hybridised with the arrays at 50ºC for 90 min. After hybridisation, alkaline phosphatase-conjugated anti-digoxigenin antibodies were used to reveal the hybridisation signals.
Results: After testing a collection of 52 strains of the ACB complex and 137 strains not belonging to the complex, both the identification sensitivity and specificity of the array were 100%. By using the array, the species distribution of 291 bacteremic isolates of the ACB complex were determined to be *A. baumannii* (221 strains, 75.9%), genomic species 3 (67 strains, 23.0%), genomic species 13 TU (2 strains, 0.7%), and unidentified *Acinetobacter* sp. (1 strain, 0.3%).
Conclusion: In conclusion, clinical isolates of the ACB complex can be quickly and accurately identified to species level by the current array. *Acinetobacter baumannii* (75.9%) was the predominant species among monomicrobial bacteraemia caused by the complex. The current method provides a rapid and accurate way to species identification of the ACB complex.

P1988 Comparison of PCR-reverse line blot and real-time PCR for the detection of dermatophytes in clinical samples

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Objectives: In a previous study PCR-Reverse Line Blot (PCR-RLB) was compared with culture and the potassium hydroxide test (KOH) for the detection of dermatophytes. PCR-RLB showed to be more sensitive than culture and KOH. Drawbacks of the PCR-RLB are the laborious nature of the test, the difficult standardisation and the interpretation of weak results. Therefore a multiplex real-time PCR was developed. The aim of this study was to compare PCR-RLB analysis with multiplex real-time PCR for the detection of dermatophytes.
Methods: Both PCR-RLB and real-time PCR targeted the ITS1 region located between the genes coding for 18S and 5.8S rRNA. The RLB membrane harboured 13 different probes to identify and discriminate between 9 different dermatophyte species. Real-time PCR consisted of two multiplex assays. One assay targeted *T. rubrum*, *T. violaceum* and *T. tonsurans*. The second targeted *Microsporum* spp., *T. interdigitale* group and the whole group of dermatophytes. Phocine herpes virus-1 was used as internal control for the real-time assays. Samples were processed using QIAamp® DNA mini kit (Qiagen, Germany) with a separate pre-lysis step.
Totally 100 clinical samples (52, 38, 10 respectively nail-, skin- and hair samples) were analysed retrospectively by real-time PCR and compared with PCR-RLB.
Results: Of the 100 samples 60 were positive with the PCR-RLB (27 *T. rubrum*, 14 *T. interdigitale*, 6 *T. tonsurans*, 3 *T. violaceum*, 1 *M. canis* and 9 *Trichophyton* spp.). All samples identified as *T. rubrum*, *T. interdigitale*, *T. tonsurans* and *T. violaceum* by the PCR-RLB were confirmed by the real-time PCR. The sample which tested positive for *M. canis* by PCR-RLB was identified as *Microsporum* spp. by real-time PCR.
The 9 samples which scored positive for *Trichophyton* spp. in the PCR-RLB yielded weak results. Of these 9 samples real-time PCR identified 3 samples as *T. interdigitale*, 1 as *T. rubrum*, 1 as *T. tonsurans*, 1 as dermatophyte positive and 3 samples remained negative.
The real-time PCR detected 8 additional samples which scored negative with the PCR-RLB. Of these 8 samples real-time PCR identified 4 samples as *T. rubrum*, 3 as *T. interdigitale* and 1 as dermatophyte positive.
Conclusion: These data show that real-time PCR is a sensitive method for detection of the most prevalent dermatophytes in nail-, skin- and hair samples. Furthermore real-time PCR is more standardised and less laborious than PCR-RLB, making it a useful tool in routine diagnostics.

P1989 Development and validation of an automated 16S rDNA assay for the detection of bacterial DNA in clinical samples

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Objectives: Since 2002 the department of Research and Development of the Laboratory for Infectious Diseases started to analyse clinical specimens on request for the presence of bacterial DNA. The assay consists of a manual DNA extraction method, followed by conventional broad-range PCR. PCR positive products are subsequently sequenced to identify the organism that was present in the clinical specimen (ref. Schuurman et al., JCM. 2004, 42;2 p734). However, the assay is very laborious and time consuming. To decrease hands on time (HOT) the extraction and detection procedure were automated. This study describes the validation of an automated 16S rDNA assay (A) for the detection of bacterial DNA in clinical samples. Method A was compared with our conventional detection method (C).
Methods: Prior to DNA extraction, samples were treated with mechanical disruption for both methods. DNA extraction was performed with the manual Boom method (C) or with the automated easyMAG specific A protocol (bioMérieux) (A). Conventional (C) and real-time (A) PCR were performed with universal primers (probe), which amplified/detected the whole 16S rRNA gene. All positive PCR products were subsequently sequenced. The analytical sensitivity of method A was assessed by dilution series of different model organisms (n = 3), spiked in phosphate buffered saline. Also, both methods were compared in a validation performed on clinical samples (n= 89).
Results: Analytical sensitivity of both methods were comparable, with limits of detection of approximately 400 – 40 CFU/PCR, 1450 – 145 CFU/PCR, and 800 – 80 CFU/PCR for Gram-negative (*E. coli*) and Gram-positive organisms (*S. aureus* and *S. pneumoniae*) respectively. Thirty eight positive and 46 negative samples were in complete concordance for both methods (94.4%). Method A detected 4 additional clinical significant positive samples (*S. intermedius*, *S. maltophilia*, *S. pneumoniae* and a mixed sequence). Method C yielded 1 additional positive result (*S. aureus*), which also was regarded clinical significant.

PCR inhibition was detected in 10% of all samples for method A in comparison with 49% for method C. Also, HOT for obtaining a PCR result decreased (~50%) when method A was applied.
Conclusions: With regards to sensitivity both methods were comparable in detecting bacterial DNA from clinical samples. However, when method A was applied HOT was reduced considerably, PCR results were easier to interpret, and inhibition rates decreased dramatically.

P1990 Novel MALDI-TOF MS based differentiation of bacteria from clinical samples: alternative to biochemical test systems ?

S. Schubert (Munich, DE)

Objective: This study compares a novel Matrix Assisted Laser Desorption/Ionisation–Time of Flight (MALDI-TOF)-based method for bacterial differentiation with common biochemical test systems. The study criteria include performance time, accuracy and easy to use of the method. An answer shall be given to whether this novel system is a future alternative to the biochemical test systems.
Methods: 600 isolates obtained form clinical samples were investigated in parallel on the MALDI-TOF system (Microflex/BioTyper, Bruker Daltonics, Bremen, Germany) and by using API® bioMérieux biochemical test system. In case of incongruent results in these systems, sequencing of 16S-rDNA genes was performed for final molecular differentiation of the isolates.
Results: 600 Isolates comprised of 200 isolates of different members of Enterobacteriaceae, 200 non-fermenting Gram-negative rods (e.g. *Pseudomonas* sp.), 80 staphylococci, 80 streptococci and 40 Gram-positive rods (e.g. corynebacteriae). The time for differentiation was about 5 min for each isolate irrespective of the group. The overall results are given in Table 1.

Table 1

	Identical results	MALDI superior	Biochemical test superior	Total no.
Enterobacteriaceae	196 (98%)	2 (1%)	2 (1%)	200
Non-fermenting Gram(−) rods	192 (96%)	6 (3%)	2 (1%)	200
Staphylococci	80 (100%)	0	0	80
Streptococci	73 (91%)	5 (6%)	2 (3%)	80
Gram(+) rods	24 (60%)	2 (5%)	14 (35%)	40

Conclusion: The MALDI-TOF based differentiation only and does not provide data on antibiotic resistance of the isolate. Moreover, the bacteria culture on plate is required and it is not suitable for detection of bacteria directly from clinical sample. The MALDI-TOF based method for bacterial differentiation provides reliable results within minutes, it is easy to perform and enables genotyping to a certain extend. Even though the initial acquisition of the MALDI-TOF apparatus is expensive, there are essentially no cost for consumables, making this system especially interesting for large scale diagnostic laboratories.

P1991 Comparison of NucliSens easyMAG and Qiagen nucleic acid extraction using respiratory specimens

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Background: The NucliSens easyMAG (bioMérieux) is an open system for automated isolation of nucleic acids (NA) from clinical samples, based upon Boom technology.
Objectives: To evaluate the performance and user convenience of the NucliSens easyMAG platform for NA extraction from respiratory specimens compared to manual Qiagen (Qia) extraction.
Materials and Methods: A total of 87 respiratory samples were included. All samples were spiked with Phocine Herpes virus (PhHV) to monitor extraction efficiency. For Qia extraction, 200µl sample was extracted according to kit instructions and eluted in 100µl. Two easyMAG (EM) protocols were applied. Throat swabs were extracted on the EM using the Generic onboard protocol with an input and elution volume of 200µl and 55µl respectively. For other sample types EM extraction was first evaluated on endotracheal aspirates (ETA) before and after pretreatment by an additional lysis step with Proteinase K for 15 min. at 56ºC. Based on this evaluation, all other samples than throat swabs were pretreated with this lysis step and extracted on the EM using the Specific onboard protocol with an elution volume of 70µl. NA extracts were analysed by real-time PCR targetting the spiked PhHV virus. Respiratory samples positive or inconclusive for *M. pneumoniae* and B. pertussis in routine with Qia extraction, were tested after EM extraction and Ct values were compared.
Results: Pretreatment of ETA by an additional lysis step increased the performance of the EM extraction from 64% to 85%. In total 78 out of 87 samples (90%) produced a comparable or lower Ct value after EM extraction compared to Qia extraction for the spiked PhHV. Ct values for *M. pneumoniae* and B. pertussis were lower in 5/6 nasopharyngeal aspirates (NPA) extracted with EM compared to Qia extraction, with a mean Ct difference of 1.9. One NPA was positive for B. pertussis after EM extraction, but negative after Qia extraction.

Sample type (n)	Number of samples with lower or equal EM Ct values (n)	Mean difference Ct Qia-EM
Total (87)	78	−0.67
ETA (13)	11	−0.12
Throat swabs (18)	17	−1.42
Sputum (10)	9	−0.67
Pleural fluid (10)	8	−0.17
NPA (16)	15	−0.66
BAL (10)	9	−0.60
Broncheal aspirate (10)	9	−0.65

Conclusion: The Nuclisens easyMAG extracted DNA from respiratory specimens more efficiently than the manual Qia method. However a proper pretreatment of these specimens is necessary. The easyMAG features user-friendly software and has a turn-around time of about 40 minutes for a total of 24 samples.

P1992 Evaluation of *E. coli*/*P. aeruginosa* PNA FISH™ and EK/*P. aeruginosa* PNA FISH™; two dual colour assays for identification of Gram-negative rods directly from positive blood culture bottles

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Objectives: The three most commonly encountered Gram-negative rods (GNR) from both community acquired and nosocomial blood stream infections are *Escherichia coli* (EC), *Klebsiella pneumoniae* (KP), and *Pseudomonas aeruginosa* (PA). Routine identifications of GNRs must await subculture and are therefore not available until the following day. Thus patients must receive broad spectrum empiric antibiotics therapy for several days, before a specific therapy can be instituted, leading to an overuse or a risk of inappropriate antibiotics therapy. *E. coli*/*P. aeruginosa* PNA FISH™ (AdvanDx) and EK/*P. aeruginosa* PNA FISH™ (AdvanDx) are new rapid fluorescence in situ hybridisation (FISH) tests using fluorescently labeled peptide nucleic acid (PNA) probes targeting rRNA targets in EC, PA, and KP. *E. coli*/*P. aeruginosa* PNA FISH™ generates green and red fluorescent signals for EC and PA, respectively. EK/*P. aeruginosa* PNA FISH™ is the same, except a green fluorescent signal indicates EC and/or KP in the sample. The tests are performed directly on smears from positive blood culture bottles and provide results within 2.5 hours.

Methods: A total of 55 routine blood culture bottles positive for GNRs were included. Smears from positive blood culture bottles were analysed by both GNR PNA FISH assays, and the results were compared to results of our routine identifications (VITEK 2).
Results: All 32 EC correctly showed green signals in both GNR PNA FISH assays. Eight KP also reacted correctly, giving green signal in the EK/*P. aeruginosa* PNA FISH assay and no signal in the *E. coli*/*P. aeruginosa* PNA FISH assay. Two PA correctly gave red signal in both assays. Thirteen isolates correctly gave no signal in both assays; 7 miscellaneous enterobacteriae, 4 anaerobes and one *Enterococcus* species (erroneous Gram smear). The sensitivities of EC, KP and PA detection were 100%, and the specificity of both assays was 100% in this study.
Conclusion: Using two PNA FISH assays we were able to identify 76% (42/55) of our blood stream pathogens. The *E. coli*/*P. aeruginosa* PNA FISH™ and EK/*P. aeruginosa* PNA FISH™ assays are rapid and accurate methods for identification of *E. coli*, *K. pneumoniae* and *P. aeruginosa* directly from positive blood culture bottles.

P1993 Microbe detection in clinical samples within a few hours by application of DNA microarrays and Lab-on-a-Chip systems

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We present a novel approach for rapid identification of infectious pathogens by combining PCR amplification with microarray analysis. In comparison to conventional diagnostic procedures, all depending on bacterial growth, this assay substantially accelerates diagnosis. For a start a classical oligonucleotide microarray (fluorescence readout) was established for the identification of 26 blood stream infection relevant pathogens. As expected from sequence similarity in the target genes (16S/18S rRNA), members of the Enterobacteriaceae family turned out to be too conserved for accurate species identification based on single species specific DNA probes. However, typical hybridisation patterns of each species could be successfully employed for accurate discrimination of each member of Enterobacteriaceae. Data from all identification experiments were collected to a hybridisation matrix which served as base for statistical analysis. The combination of rank normalisation and k-nearest-neighbour method enabled a correct determination of 100% of the bacteria at genus level and 96.7% at species level. After thorough assay optimisation (DNA isolation, PCR amplification, PCR target labeling) we succeeded to identify 10 *E. coli* cells per mL whole blood. A diagnostic chip for the identification of kolpitis causing pathogens including bacteria, yeasts, moulds, parasites and viruses is currently under development. Up to now a microarray was established which can identify kolpitis relevant bacteria and yeasts down to species level. Probe validation showed good agreements with conventional identification methods and further probes are already introduced for the detection of intracellular pathogens. Relying on our experiences from pathogen microarrays we currently develop a Lab-on-a-Chip (LOC) device aiming to miniaturize and accelerate the diagnostic assay further.

P1994 Application of molecular techniques for the diagnosis of community-acquired bacterial meningitis in nine Spanish hospitals

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Objective: To evaluate the sensitivity of three real-time polymerase chain reaction (RT-PCR) assays for the diagnosis of community-acquired bacterial meningitis (Ca-ABM), compared to Gram staining and culture.
Methods: Three different RT-PCR assays were performed in cerebrospinal fluid (CSF) and/or blood of all patients with Ca-ABM diagnosed in nine hospitals of the Spanish Network for Investigation in Infectious Pathology (REIPI). Two multiplex RT-PCR with two different detection instruments TaqMan system were used for the detection of: ctrA from *Neisseria meningitidis*, ply from *Streptococcus pneumoniae* and bexA from *Haemophilus influenzae* type b. The third RT-PCR was based on amplification of 16S rDNA gene and when is necessary, the amplification product was revealed with DNA-chips by hybridisation. Gram staining, CSF and blood culture were performed by conventional methods.
Results: 415 patients were diagnosed of Ca-ABM. Were performed 412 Gram staining, 414 culture and 250 RT-PCR on CSF samples. Obtaining positive results in 231 (56.2%), 295 (71.3%) and 185 (74%) respectively. In blood were performed 410 cultures and 149 RT-PCR, where 214 (55.6%) and 59 (39.6%) were positive. In 134 patients all diagnosis procedures were performed simultaneously. In CSF, Gram staining showed a sensitivity of 63.4%, culture 73.1% and RT-PCR 74.6% and in blood culture had a sensitivity of 57.5% and the RT-PCR 38.8%.Pneumococcal meningitis was diagnosed in 160 (38.6%) patients. All diagnosis procedures were done in 57 with the follow sensitivity: CSF Gram staining 80.7%, CSF culture 86% and CSF RT-PCR 93%, blood culture 66.7% and blood RT-PCR 43.9%. Five CSF samples showed negative culture whereas RT-PCR was positive and 1 sample showed positive Gram staining and culture with negative RT-PCR. Meningococcal meningitis was diagnosed in 109 (26.3%) patients; 43 patients with all diagnosis procedures. CSF Gram staining had a sensitivity of 65.1%, culture 72.1% and RT-PCR 90.7%. Blood culture showed a sensitivity of 44.2% and RT-PCR 51.2%. Thirteen CSF samples showed negative culture whereas RT-PCR was positive and 3 had positive culture with negative RT-PCR.
Conclusions: Gram staining is a faster, economic and specific procedure. RT-PCR showed a slightly better sensitivity than culture in CSF whereas worse results were obtained in blood, however is appropriate when lumbar puncture is contraindicated. CSF culture is necessary to perform typing and susceptibility studies.

P1995 MALDI-TOF MS as a universal tool for the characterisation of pathogenic bacteria – A comparative analysis of MALDI-TOF MS spectral fingerprints over a six-year period

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Objective: There has been a significant increase in the number of papers demonstrating MALDI-TOF MS as a powerful, rapid, high throughput technique for the characterisation of pathogenic organisms. These isolate are generally characterised against a limited database built in house with the experimentation carried out in relatively quick succession, on the same sample set and same instrumentation by the same operator. In the majority of these cases identification is based upon a small number of exclusive peaks associated with each species, since variability is often observed in the spectral pattern. This paper examines this variability and hence the ability of the technique for use as a universal tool.
Method: Six strains, *Bacteroides fragilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Corynebacterium jeikium*, *Vibrio parahaemolyticus* and Micrococcus lylae were analysed by MALDI-TOF MS repeatedly over a period of 6 years. The resulting spectral fingerprints were derived from isolates cultured from different freeze dried ampoules; media; reagent stocks; instrumentation and technical personnel. The resulting spectra patterns were compared for reproducibility and the effects upon identification against an extensive MALDI-TOF MS database of over 5000 spectral entries, covering more than 600 different species examined.
Results: The results demonstrate the spectral fingerprints are very robust, with variations generally observed in the peak intensity of the mass patterns. Furthermore the differences observed were insufficient to cause misidentification of the spectral fingerprint against a substantial database.
Conclusion: This extended study therefore validates MALDI-TOF MS as a tool for rapid universal characterisation of bacteria.

P1996 ***Abiotrophia defectiva*** infection of a total hip arthroplasty diagnosed by 16S r RNA sequencing

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Objective: *Abiotrophia defectiva* is a fastidious Gram-positive bacterium that is known for its pleiomorphic coccobacillar forms and that may appear as streptococci. Previously it was referred to as part of the nutritionally variant streptococci. Nowadays it is the single species of the *Abiotrophia* genus. It is a resident of human oral cavity and urogenital and intestinal tracts and accounts for approximately 5% of cases of bacterial endocarditis. There have been some sporadic reports of *A. defectiva* as infective agent of osteoarticular infections and we describe the first case of a total hip arthroplasty infection caused by *A. defectiva*.

Methods: A 71-year-old woman was admitted to our hospital for replacement of her left hip arthoplasty that she had undergone 2 years earlier in another hospital. She presented with a history of increasing pain in her left upper leg and knee. She had no fever. Progressive osteolysis near the prothesis was shown on consecutive radiographs and confirmed by scintigraphy. She was diagnosed with an arthroplasty infection.

Cultures of bone fragments and of pus near the prothesis showed only growth in broth media and on the plate used to detect anaerobes which among others contains horse blood and cysteine. Gram stain revealed a pleiomorphic Gram-positive coccobacillus that could not be identified by biochemical techniques. 16S rRNA sequencing was performed and showed 99% homology with A. defectiva sequences. Retrospectively the Gram stain matched the species.

Conclusion: Since its pleiomorphic appearance and relatively infrequent incidence, *A. defectiva* is difficult to identify. It mimics Gram-positive rods and therefore might have a place in algorithms used to identify this group of bacteria. Standard biochemical techniques often fail to identify this micro-organism. 16 S rRNA sequencing offers a good methodology to recognize this bacterium.

The proposed affinity of *A. defectiva* for avascular tissue is underlined by this first report of a hip arthoplasty infection. Considering the increasing use of foreign body material in modern medicine and the growing us of molecular diagnostics, one might expect a rising incidence of *A. defectiva* associated infections.

P1997 Evaluation of an acromopeptidase based extraction method and use of the Rotor-Gene to improve the BD GeneOhm IDI MRSA assay

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Objectives: The IDI MRSA assay was first implemented at the RFH in 2006. Its use has enabled the timely implementation of appropriate infection control measures, which have contributed to the reduction in MRSA rates within the Trust. Currently the test is only used for specific patient groups, however there is the desire to increase the number of patients screened using this methodology. Due to the hands on time required for the IDI MRSA assay, increasing the numbers would have an effect on the time required to perform each run and would impact on turnaround times. We therefore set out to evaluate the use of an acromopeptidase extraction method (ACP) which requires less hands on time, the Corbett Research CAS-1200 to automate the PCR set up and the Corbett Research Rotor-Gene platform to increase the capacity of each run.

Methods: Over a five week period a preliminary study was performed during which 511 samples were tested using the routine method (IDI extraction and the SmartCycler, method 1) and three other methods: ACP and the SmartCycler (method 2); IDI extraction and the Rotor-Gene (method 3); and ACP and the Rotor-Gene (method 4). In-house cut-off values were used to analyse the Rotor-Gene data. Samples that were from known MRSA positive patients, which were culture positive and/or positive by all four methods, were regarded as true positive samples.

Results: During the preliminary study the inhibition rates were 35 (6.8%), 37 (7.2%), 9 (1.8%) and 6 (1.2%) for methods 1–4 respectively; the false positive rates were 5 (1%), 1 (0.2%), 1 (0.2%) and 2 (0.4%) for methods 1–4 respectively and the false negative rates were 4 (0.8%), 10 (2%), 6 (1.2%) and 7 (1.4%) respectively.

Conclusions: This preliminary study has shown that an ACP extraction method and the Rotor-Gene could be used to improve the workflow of the IDI MRSA assay without compromising the sensitivity or specificity of the assay. The lower inhibition rates observed when using the Rotor-Gene were a reflection of the less stringent in-house cut-off values used. Subsequent to the study additional measures have been put in place and the inhibition rate of the routine method has now been reduced to approximately 2%. To fully validate the use of ACP and the Rotor-Gene a further 2000 samples are in the process of being tested and appropriate cut-off values are being established.

P1998 Simple methods to screen efflux pump activity and efflux pump inhibitors in multidrug-resistant *Escherichia coli*

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Objectives. Over-expression of efflux pumps (EPs) in Gram-negative bacteria is associated with Multi-Drug Resistance (MDR). EPs inhibitors (EPIs) can be used to detect the activity of EPs and to reverse efflux-mediated MDR. In this study, 3 simple methods were developed to quantify efflux in MDR *E. coli* and screen for effective EPIs.

Methods. Thirty veterinary *E. coli* strains with MDR phenotype (resistance to 2 or more classes of antibiotics) were evaluated for ethidium bromide (EB) efflux activity by an agar-based method. Strains that showed efflux activity were selected for the screening of EPIs by the microplate method. Briefly, antibiotic discs were placed in 24-well plates containing media with/without the following EPIs (at half their MIC): Phe-Arg-napthylamide (PAN), thioridazine (TZ), chlorpromazine (CPZ) and carbonyl cyanide m-chlorophenylhydrazone (CCCP). After 16 h, wells were examined for absence (reversal of resistance), partial (reduction of resistance) or full growth (no effect). Strains that showed reduction or reversal of resistance were then evaluated for efflux with/without EPIs by a semi-automated real-time fluorometric method. Expression of EPs genes was assayed by RT-PCR.

Results. The EB agar method correctly identified 4 strains with increased EPs activity, which was further quantified by the fluorometric assay. This efflux activity was reduced or even reversed to clinical levels by the EPIs PAN and TZ. The RT-PCR assay of these strains showed an increased level of expression of their major EPs.

Conclusion. The methodologies developed and to be described in detail can be used for detection of MDR strains whose phenotype and gene expression are consistent with increased efflux activity as well as for the screening of agents for EPI activity against collections of MDR clinical isolates. These methods are expected to be eventually used in the clinical bacteriology laboratory.

P1999 Relevance of testing for microbial DNA in critically ill patients

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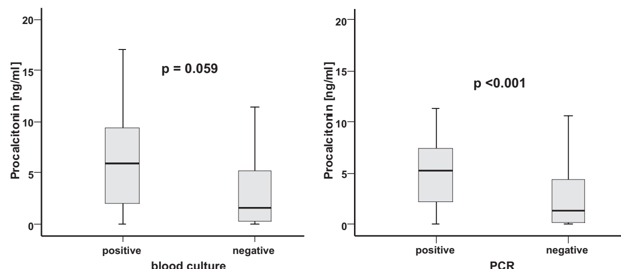
Objectives: Blood culture is an important method for identifying the underlying microorganism causative of sepsis. However, blood culture results may take up to 48 hours possibly delaying appropriate antibiotic therapy. The goal of this study was to evaluate the performance of microbial DNA testing in the blood compared to blood culture in critically ill patients.

Methods: The study was performed in 3 centres. Inclusion criteria were either severe sepsis (sepsis group) or elective surgery without evidence of infection (control group). A blood culture (BC) was obtained and 10 ml EDTA blood were simultaneously taken by sterile venous puncture.

Further samples were taken on max. 3 consecutives days. Microbial DNA was measured by PCR with the LightCycler System 2.0 (Roche Diagnostics).

Results: 142 sepsis patients (236 samples) and 63 control patients (111 samples) have been included. Control patients had 3.6% positive PCRs and 4.5% positive BCs (sensitivity 0; specificity 0.95). 34.7% of PCRs were positive in the sepsis group compared to 16.5% of BCs (sensitivity 0.79; specificity 0.74). Procalcitonin values at diagnosis of sepsis were higher in PCR/BC positive than in PCR/ BC negative patients (figure).



Conclusion: Concordance between blood culture and PCR results is very good in patients without evidence of infection. In patients with sepsis, PCR showed positive results more often than the blood culture. PCR-based culture-independent detection correlated well with disease severity as reflected in plasma procalcitonin levels. In the light of shorter time to result, PCR based detection might trigger appropriate antibiotic therapy in the early therapeutic window which determines prognosis of the patient with severe sepsis.

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P2000 Evaluation of the Hyplex®MBL ID multiplex PCR-ELISA system for direct detection of blaVIM and blaIMP genes in blood and other clinical material

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Objectives: Hyplex®MBL ID Multiplex PCR-ELISA (BAG Health Care, Germany) is a new diagnostic method for the direct detection of metallo-β-lactamase (MBL) genes of the VIM and IMP types in clinical specimens. The method was tested in Greek hospitals, characterised by high prevalence of MBL-producing isolates.

Methods: The hyplex®MBL ID method involves amplification of blaVIM/blaIMP bacterial DNA by multiplex PCR and hybridisation of the PCR products to specific oligonucleotide probes in an ELISA-based format. The method was applied to 326 samples obtained during September 2007 in three Athens hospitals. These included 90 positive blood culture bottles containing at least one Gram-negative species and 236 consecutively collected samples including urine (n = 60), pus swabs (n = 91) and bronchial secretions (n = 85). Results were compared to those of a blaVIM/IMP PCR screening of the isolated Gram-negative bacteria.

Results: Nineteen of the 90 blood cultures were positive by the hyplex®MBL ID. blaVIM-1- or blaVIM-2-carrying isolates (8 *Pseudomonas aeruginosa*, 9 *Klebsiella pneumoniae*, 1 *Providencia stuartii* and 1 *Enterobacter aerogenes*) were isolated from all hyplex-positive samples. blaVIM-carrying isolates were not identified in any of the hyplex-negative samples (sensitivity and specificity 100%). Five of the urine samples containing blaVIM carriers (2 *P. aeruginosa* and 3 *K. pneumoniae*) were positive by the hyplex®MBL ID; MBL-positive organisms were not detected from 2 hyplex-positive samples. Eight of the 9 pus samples with VIM-producers (5 *P. aeruginosa*, 2 *K. pneumoniae* and 1 *E. cloacae*) were also positive by hyplex®MBL ID; one sample appeared hyplex-positive but MBL producers were not isolated; blaVIM was also identified in 1 hyplex-negative *P. aeruginosa* isolate. In respiratory samples the hyplex®MBL ID method classified as positive 40 samples containing VIM-producers; MBL producers were not isolated from 2 hyplex-positive respiratory samples. Sensitivity and specificity of the hyplex®MBL ID system for specimens other than blood were 98% and 97%, respectively.

Conclusion: The hyplex®MBL ID test can be reliably applied for the timely detection of blaVIM carriage by Gram-negative species in positive blood cultures in this setting. The assay is also efficient in detecting MBL genes in other clinical specimens however, the diagnostic utility of this approach remains to be evaluated.

P2001 Development of a real-time PCR assay for the specific detection of Arcobacter spp.

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Objectives: Arcobacters are fastidious, Gram-negative, non-sporing, motile, spiral-shaped organisms that are considered emerging food-borne pathogens producing enteritis and septicaemia. Routine identification is difficult, and it is frequently misidentified as atypical *Campylobacter* when relying on conventional plating methods and phenotypic tests, what may lead to a substantial underestimation of their true incidence. Therefore the objective of this study was the development of a rapid and reliable real time PCR method to target the emerging pathogen Arcobacter sp.

Methods: A. butzleri DSM 8739, A. cibarius DSM 17680, A. cryaerophilus DSM 7289 and A. skirrowii CIP103588 were used as reference strains to set up the assay. A test panel of 15 collection strains from different species and genus were also included to study the specificity of the assay.

The optimal conditions for the real time PCR were established by testing different temperatures, MgCl2 and BSA concentrations, and product extension times. The primer's set detected a 331-bp fragment of 23S rRNA gene of Arcobacter sp. Each reaction mixture consisted of 2 µl of DNA, 0.5 µM of each primer, 2mM MgCl2 and 2 µl of LightCycler Fast-Star DNA Master SYBR Green I Mix in a reaction volume of 20µl. The reaction was performed in a LightCycler 2.0 (Roche Applied Science®) with a preliminary denaturation for 10min at 95ºC (slope 20ºC/s), followed by 40 cycles of denaturation at 95ºC for 10s (20ºC/s), annealing at 58ºC for 4s (20ºC/s) and primer extension at 72 ºC for 14s (20ºC/s), with a single fluorescence acquisition step at the end of the extension. This was followed by melting point analysis of the DNA product with continuous fluorescence acquisition. A final cooling step was performed at 40ºC for 30s (20ºC/s). All the assays were performed by duplicate.

Results: The real time PCR assay resulted in the expected 331-bp amplified DNA fragment from all Arcobacter reference strains, with a melting temperature ranging from 84 to 84.9ºC, and a range of Ct values from 10.89 to 22.60 for different DNA concentrations. No amplification was observed when any strains different from Arcobacter spp. were tested.

Conclusion: The real time PCR assay described in this study provides a rapid and specific method to detect Arcobacter spp. and it could be very useful for assessing Arcobacter contamination in environmental samples, evaluating the risk of transmission via food chain and its potential as Public Health Hazard.

P2002 Pili distribution among *Streptococcus pneumoniae* associated with invasive disease in Portugal

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Objective: Evaluate the distribution of the pilus islet among *Streptococcus pneumoniae* isolated from invasive infections

Methods: Pilus-like structures were recently recognised in pneumococci and implicated in the virulence of this bacterium. These structures are encoded by the genes of the rlrA islet, and signature tagged mutagenesis studies implicated the rlrA gene, encoding a putative transcriptional regulator, as an essential virulence gene in murine models

of infection. Nevertheless, the rlrA islet is not universally distributed among pneumococcal strains.
To evaluate the distribution of the pilus islet, we determined the presence of the rlrA gene as a marker for the locus, among a collection of invasive isolates recovered in Portugal between 1999 and 2002, and analysed its association with antibiotic resistance, capsular serotypes, clusters defined by pulsed-field gel electrophoretic profiles (PFGE) and multilocus sequence types.
Results: A minority of the isolates were positive for the presence of the rlrA gene (27%). Nevertheless, serotype distribution analysis showed a high correspondence between the serotype and the presence or absence of the rlrA gene (Wallace coefficient, W=0.778). Additionally, even within serotypes, variation of the presence of the pilus islet between PFGE clones was observed. A higher Wallace coefficient between PFGE clones and the presence of the locus (W=0.939) indicates that the presence of the islet is a clonal property of *S. pneumoniae*. Analysis of rlrA negative isolates revealed heterogeneity in the genomic region downstream of the pfl gene, the region where the islet is found in other isolates, compatible with recent loss of the islet in some lineages.
Conclusion: The low prevalence of the rlrA islet in our collection of invasive isolates indicates that pili are not essential virulence factors in human infections and that their potential use in a vaccine would offer limited benefits. However, its association with a widely disseminated pneumococcal clone (Spain9V-3) may indicate that pili could facilitate dissemination or colonisation of the host, as suggested recently. Nevertheless, since serotype prevalence is changing as a consequence of vaccine introduction, the acquisition of the pilus islet by emerging clones or the increase in incidence of clones carrying the rlrA islet may clarify the role of this surface structure in human infections.

Molecular biology of β-lactamases and other resistance genes. Plasmids, integrons etc

P2003 Complete nucleotide sequences of plasmids pEK204 and pEK516, encoding CTX-M enzymes in two major UK *Escherichia coli* strains

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Objectives: *E. coli* strains with the CTX-M-15 ESBL have become prevalent in the UK since 2003. They include 5 ancestrally-related strains, A-E: A is the UK's most prevalent ESBL-producing *E. coli* strain and has the plasmid pEK499 (117,536-bp), sequenced previously; D is local to one site; B, C and E are scattered. Some strain C isolates produce CTX-M-3, rather than CTX-M-15 enzyme. We sequenced the blaCTX-M-3 and blaCTX-M-15-harbouring plasmids pEK204 and pEK516 from strain C and D representatives, respectively, and compared them with pEK499 of strain A.
Methods. Plasmids pEK204 and pEK516 were transferred by conjugation into *E. coli* J-53. Randomly sheared plasmid fragments were cloned into pGEM-Teasy vector and transformed into *E. coli* DH10b. Inserts were sequenced by dye terminator chemistry. Sequences were assembled using the Staden Package. Combinatorial PCRs, directed PCRs, and walking reads were used to assemble the sequences and to fill-in gaps.
Results. Plasmid pEK516 (64,471-bp, strain D) harboured 103 predicted open reading frames, with 7 antibiotic resistance genes – aac6′-Ib-cr, aac3-IIa, blaCTX-M-15, blaOXA-1, blaTEM-1, catB4 and tet(A) – clustered in a 22-kb region. Plasmid pEK204 (93,732-bp, strain C) carried only blaCTX-M-3 and blaTEM-1. Both were conjugative IncFII plasmids, but variations in their oriV sequences suggested that they belonged to different sub-types. blaCTX-M genes were linked to an ISEcp1 element in both cases, with a 128-bp link for blaCTX-M-3 on pEK204 and a 48-bp link for blaCTX-M-15 on pEK516. Plasmid pEK516 shared 75% of its DNA sequence with pEK499, albeit with considerable rearrangements. Plasmid pEK204 showed only c. 10% homology with these plasmids.
Conclusion. Plasmid pEK516 (strain D) was highly related to plasmid pEK499, which encodes CTX-M-15 enzyme in epidemic strain A, but was 53-kb (45%) smaller, encoded gentamicin resistance, and was conjugative. Plasmid pEK204 (strain C) was very different and had a longer ISEcp1-blaCTX-M link, as in the Polish plasmid, pCTX-M-3. We conclude that ancestrally-related *E. coli* strains have acquired different plasmids encoding CTX-M ESBLs or separate occasions.

P2004 High prevalence of blaCTX-M-14 in Spain is mainly associated with the dissemination of an epidemic plasmid belonging to IncI complex

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Objectives: CTX-M-14-producing isolates have sporadically been described in some Asian, American and European countries but is one of the most prevalent ESBLs in China, Canada and Spain. We described the molecular characterisation of Spanish CTX-M-14 producing isolates in order to understand their recent dissemination in our country.
Methods: We analysed 63 isolates from patients (16 hospitalised/17 non-hospitalized), 24 healthy volunteers and 2 food samples (2000–2005). Clonal relatedness was established by XbaI-PFGE, MLST and identification of phylogenetic *E. coli* groups. Transfer of blaCTX-M-14 was searched by broth and filter mating. Location of blaCTX-M-14 genes was assessed by hybridisation of I-CeuI digested genomic DNA with specific probes (rep, blaCTX-M-14, 16S rDNA). Plasmid characterisation included detection of size and content (S1 nuclease), identification of incompatibility groups by PCR replicon-typing and the rep-cop region and comparison of RFLP patterns. Genetic environment of blaCTX-M-14 was characterised by screening the presence of sequences associated with blaCTX-M-14 as ISEcp1, ISCR1, and further PCR overlapping assays based of known sequence structures.
Results: CTX-M-14 producing isolates were mostly recovered from the community (74.6%) and associated with urinary tract infections (60.3%). Most isolates were identified as *E. coli* (98%) which were clonally unrelated by PFGE and correspond to different phylogenetic groups: A (37%), D (34%), and B1 (29%). MLST analysis revealed diversity of ST among *E. coli* phylogroups D and B1 while isolates of phylogroup A mostly belonged to ST10. blaCTX-M-14 was located in plasmids from 45 to 330kb and transferred by conjugation in most cases (75%). The ISEcp1-blaCTX-M-14-delta IS903 platform was identified on a 80kb plasmid belonging to the IncI complex (cop-rep region identical to that of pR387 which was traditionally classified as IncK). In three isolates (2002–03), blaCTX-M-14 was associated with a class 1 integron bearing ISCR1(In60) as happen for blaCTX-M-9, suggesting recent evolution from blaCTX-M-9 to blaCTX-M-14.
Conclusions: CTX-M-14 is associated with *E. coli* causing urinary tract infections in the community. Recent increase of CTX-M-14 is mainly associated to the spread of an epidemic plasmid belonging to the IncI complex although other genetic environments also endemic in our country might contribute to further spread and persistence.

P2005 International dissemination of a multi-resistant IncA/C2 plasmid containing blaTEM-24, Tn21 and Tn1696 among epidemic and non-epidemic Enterobacteriaceae species

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Objectives: TEM-24 is a widely disseminated ESBL mostly associated with epidemic *Enterobacter aerogenes* and *Klebsiella* spp. strains. The aim of this study was to analyse clonal and plasmid diversity among TEM-24 producing strains from 4 European countries.
Methods: Twenty eight TEM-24-Enterobacteriaceae isolates from Portugal, Spain, France and Belgium identified as *E. aerogenes* (n = 13), *Escherichia coli* (n = 6), *Klebsiella pneumoniae* (n = 6), *Proteus mirabilis* (n = 2) and *Klebsiella oxytoca* (n = 1) were studied (1998–2004). Only one isolate/patient was included. Clonal relatedness was established by PFGE and *E. coli* phylogenetic groups were searched by PCR. Antibiotic susceptibility testing and conjugation assays were performed

using standard methods. Plasmid analysis included determination of size (Barton's method), incompatibility groups (rep-PCR, hybridisation, and sequencing) and comparison of RFLP patterns. Characterisation of integrons (class 1, 2 and 3), transposable elements and sul genes was performed by screening of sequences related to these elements (orf5, orf6, IS1326, IS1353, IS6100, tnpA, tnpR, tni, and mer), PCR overlapping based on Tn21 and Tn1696 sequences, and further sequencing.
Results: *E. aerogenes*, *K. pneumoniae* and *P. mirabilis* isolates corresponded to single PFGE types while *E. coli* were clonally unrelated (phylogroups D and A), all containing a 170–190kb-IncA/C2 conjugative plasmid carrying blaTEM-24 and conferring resistance to kanamycin, tobramycin, chloramphenicol, trimethoprim and sulfonamide. Different multi-resistant sequences were identified: i) In4 integron (aacA4) within Tn1696, ii) In0 derivative (dfrA1-aadA1) within Tn21 sequences, and iii) Tn3.
Conclusions: International dissemination of TEM-24 is associated with epidemic strains and an IncA/C2 epidemic plasmid containing multiple and genetic platforms conferring a multi-resistant phenotype. Interestingly, this trait was also conserved over the studied period.

P2006 Recent dissemination of blaTEM-52-producing Enterobacteriaceae in Portugal is caused by spread of IncI plasmids among *Escherichia coli* and *Klebsiella* clones

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Objectives: Since its first description in 1996, TEM-52 has been sporadically identified in Europe, Korea and Canada among Enterobacteriaceae from both human and animals. An epidemic IncI1 plasmid containing blaTEM-52 has been recently identified among *Salmonella enterica* in France. We analysed the diversity of TEM-52 producing isolates from Portugal in order to understand the reasons for recent spread in our country.
Methods: Twenty one TEM-52-producing isolates recovered from Portugal (n = 19 *Escherichia coli*; n = 2 *Klebsiella pneumoniae*) between 2002 and 2005 were studied. The isolates were obtained from hospitals (n = 7), poultry (n = 12) and sewage (n = 2). Only one isolate/phenotype was included. Clonality was established by PFGE and *E. coli* phylogenetic groups by PCR as reported. ESBL identification was achieved by IEF, PCR and sequencing. Standard methods were used for conjugation assays as well as antibiotic susceptibility testing. bla location was accessed by hybridisation of I-CeuI-digested DNA with an intragenic blaTEM-52 probe. Plasmid characterisation was accomplished by the Barton's method, identification of plasmid incompatibility groups by PCR, hybridisation and sequencing, and analysis of RFLP patterns. Presence of sul genes and class 1 and 2 integrons was searched in wild-type strains by PCR.
Results: High clonal diversity was found among studied isolates (n = 21 PFGE-types). Most *E. coli* clinical isolates were assigned to phylogenetic group B1. *E. coli* recovered from poultry and sewage were identified as phylogroups A (4 A0 and 3 A1), B1 (n = 2) and D (3 D1 and 1 D2). Conjugation was achieved in 71% of the isolates. blaTEM-52 gene was carried by two different plasmids of approximately 90kb and 30kb. They contain sequences characteristic of incompatibility groups IncI1 (90kb) and IncK (30kb), both belonging to the IncI plasmid group. All isolates harboured one or more sul genes: sul1 (47%) mainly associated with class 1 integrons, sul2 (65%) and sul3 (35%) which was mainly present in poultry isolates.
Conclusions: Wide and recent dissemination of blaTEM-52 among *E. coli* and *K. pneumoniae* strains from Portugal seems to be associated with IncI plasmids rather than clonal expansion.

P2007 Characterisation of plasmids harbouring qnrS1, qnrB2 and qnrB5′ genes in Dutch quinolone-resistant *Salmonella* isolates

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Objective: The aim of this study was to identify and characterize plasmids harbouring qnr genes in quinolone resistant *Salmonella* isolated in The Netherlands. Recently the first qnrB and qnrS genes were described in isolates from patients and a broiler chicken from Netherlands. QnrS1 was predominantly associated with serovar Corvallis (25 strains), but it was also found in serotype Kentucky, Saintpaul, Anatum, Montevideo and Stanley. QnrB2 was detected in one strain of S. Bredeney and qnrB5′ in one strain of S. Typhimurium. Whereas plasmid-mediated quinolone resistance of the qnr type is emerging in Enterobacteriaceae worldwide, it remains rare in *Salmonella*. These genes had previously been described in very few strains of *Salmonella* in collections from USA, France and UK. The heterogeneity of the strains indicates that the qnr genes are disseminated by plasmids but little information is available on the type of plasmids carrying the different qnr gene variants. Currently, there is only one small plasmid (10066 bp) of the ColE family identified in a S. Typhimurium strain in UK.
Methods: Plasmids were transferred by conjugation or transformation to a recipient *E. coli* K12 strain by standard methods. In the transconjugants and transformants plasmids were categorised in Incompatibility groups (Inc) or families by the PCR-based replicon typing and analysed by RFLP. The β-lactamase blaLAP-2 was identified by PCR and sequencing.
Results: the qnrS1 gene was located on three kinds of plasmids, one type of approximately 45–60 kb belonging to IncN identified in three different *Salmonella* serotypes and one type consisting of small ColE plasmid derivatives, identified in S. Corvallis, Anatum and Montevideo strains. The third type of plasmid carrying qnrS1 was >205 kb, belonging to the IncHI2 group, and positive for blaLAP-2 gene. This plasmid was identified in a S. Stanley strain. Both qnrB2 as qnrB5′, were located on conjugative IncN plasmids.
Conclusions: The molecular epidemiology of the qnr genes in Enterobacteriaceae is explained by efficient vertical and horizontal transmission of these genes mediated by mobile genetic elements and plasmids. The possibility to rapidly identify and recognize plasmid scaffolds associated to the different qnr gene variants, assigning them to specific families is very helpful to better trace the epidemiology and spread of these variants in isolates from humans, environment and animal sources.

P2008 blaVEB-6, a novel ESBL gene in a complex structure

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Objectives: To characterize a blaVEB gene and its genetic context from a local *Proteus mirabilis* isolate (JIE273) resistant to 3rd generation cephalosporins.
Methods: JIE273 was identified as an ESBL-producer using the keyhole disc approximation test. A blaVEB gene, but not blaCTX-M, blaTEM, blaSHV, or blaGES genes, was detected by PCR and sequenced. Mating experiments and electroporation were performed to try and obtain a blaVEB-carrying plasmid from JIE273. The blaVEB gene was cloned into pBBR1-MCS1 with and without the predicted promoter provided by a adjacent repeated element (Re1). The MICs of various β-lactams against transformants containing pBBR:VEB6 (blaVEB-6 alone) and pBBR:VEB6pro (blaVEB-6 with Re1 promoter) were determined by E-test. The genetic context of blaVEB-6 was determined by conventional and long-range PCR.
Results: Sequencing of the blaVEB gene in JIE273 revealed a A53G point mutation compared with blaVEB-4, the closest match, predicting one amino acid substitution (I18V) in the leader peptide. This variant was designated blaVEB-6. Despite repeated attempts, mating and electroporation with JIE273 plasmid preparations were unsuccessful, suggesting a possible chromosomal location for blaVEB-6. An *E. coli*

transformant containing pBBR:VEB-6 was resistant to ceftazidime (CAZ, MIC=32 μg/mL), but not to cefotaxime (CTX, MIC=0.032) and cefepime (CFP, MIC=0.075), indicating VEB-6 is a ceftazidimase. However, an *E. coli* transformant containing pBBR:VEB-6pro was resistant to CAZ (MIC > 256), CTX (MIC > 32) and CFP (MIC > 32), indicating the strong activity of the promoter provided by Re1. blaVEB-6 was found in a similar genetic context to some other blaVEB genes, associated with repeated elements (Re1 and Re2) and flanked two copies of 3′-CS. One 3′-CS was adjacent to a cassette array containing a novel combination of gene cassettes (Figure 1).

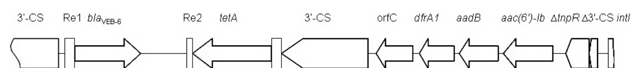

Figure 1.

Conclusion: A novel blaVEB-type variant, blaVEB-6, has been detected in Australia. Its product, VEB-6, is a ceftazidimase, but in the presence of a strong promoter conferred additional resistance to CTX and CFP. The two Re and/or the two copies of the 3′-CS flanking blaVEB genes might be involved in the spread these genes.

P2009 Genetic organisation of transposase regions surrounding blaKPC carbapenemase genes on plasmids from *K. pneumoniae* and *E. cloacae*

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Objectives: We previously described several carbapenem-resistant (CR) *K. pneumoniae* from the NY-Presbyterian Hospital in NYC encoding KPC carbapenemases. Such strains are spreading to other regions of the US, South America, and the Middle East. We have determined the sequence of the flanking regions adjacent to the blaKPC and transposon genes of *K. pneumoniae* and *E. cloacae* isolates to better elucidate the mechanism of kpc transfer in these isolates.
Methods: Plasmids encoding the blaKPC gene were isolated from several CR isolates using high speed Maxi kits and characterised by standard restriction enzyme analysis. Plasmids (65 to 78 Kb) from non-clonal strains (PFGE) were purified and submitted for total DNA sequencing.
Results: Comparison of two plasmids from CR isolates revealed a conserved 10 kb region representing a probable mobile element of high G+C content (~62%). The region contains genes for three transposases and two transposase-associated proteins, the blaKPC, and a 67% G+C XerC-like recombinase gene that is often part of integrative and conjugative elements (ICE elements). The genetic environment of the element containing blaKPC-3 in the *E. cloacae* isolate indicated insertion adjacent to DNA containing a disrupted copy of a Tn3 transposase with nearly 100% identity to a penicillin resistance associated *Haemophilus influenzae* ICE element and, further downstream, plasmid conjugal transfer and transposase genes. A tnpR resolvase gene and the aad4 was located 11,000 bp upstream of blaKPC3. Further sequencing of the *E. cloacae* plasmid indicated the presence of a class I integron, but the blaKPC and flanking transposase genes did not appear to be part of this structure. Characterisation of the second plasmid from a CR *K. pneumoniae* encoding bla KPC-2 revealed an identical 10 kb region, but in this case the element disrupted a maturase gene from a group II self-splicing intron.
Conclusion: Endemic outbreaks of CR *K. pneumoniae* encoding KPC carbapenemases in NYC hospitals has become more common and this resistance gene is now found in many other species of Enterobacteriaceae and in *Pseudomonas aeruginosa* in several other parts of the world. Our data agree with previous studies indicating that portions of transposase genes flank the kpc structural genes located in plasmids from *K. pneumoniae*. In addition, we have found that some of these transposase genes are intact and in some cases may be part of more complex genetic elements.

P2010 Genetic structures at the origin of acquisition of the β-lactamase blaKPC gene

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Objectives: Emergence and dissemination of Enterobacteriaceae isolates harbouring carbapenemases is a significant threat to the management of nosocomial infections. The Ambler class A carbapenem-hydrolysing KPC β-lactamase, which hydrolyse all β-lactams except cephamycins, are increasingly reported in enterobacterial species, mostly in *Klebsiella pneumoniae* initially from the US (especially from New York City hospitals) but also from other countries from different continents, other enterobacterial species or from *Pseudomonas aeruginosa*. The aim of this work was to investigate the molecular determinants for this rapid spread.
Methods: Genetic structures surrounding the carbapenem-hydrolysing Ambler class A blaKPC gene were characterised by cloning, PCR analysis and sequencing in several KPC-positive *K. pneumoniae* and *P. aeruginosa* isolated from the US, Colombia and Greece. The strains were analysed for their DNA content (genomic or plasmid) by plasmid extraction, conjugation and electroporation assays.
Results: The blaKPC genes were associated with transposon-related structures. In *K. pneumoniae* YC isolate from the US, the β-lactamase blaKPC-2 gene was located on a novel Tn3-based transposon, Tn4401. Tn4401 was 10 kb in size, delimited by two 39-bp imperfect inverted repeat sequences, and harboured in addition to the β-lactamase blaKPC-2 gene, a transposase and a resolvase gene, and two novel insertion sequences, ISKpn6 and ISKpn7. Tn4401 has been identified in all isolates. Two isoforms of this transposon were found, Tn4401a found in *K. pneumoniae* YC and *K. pneumoniae* GR, from the US and Greece, respectively differed by a 100-bp deletion, located just upstream of the blaKPC-2 gene, as compared to Tn4401b found in the Colombian isolates. In all tested isolates, Tn4401 was flanked by a 5-bp target site duplication, signature of a recent transposition event, and was inserted in different ORFs located on plasmids varying in size and nature.
Conclusion: Tn4401 is likely at the origin of the carbapenem-hydrolysing β-lactamase KPC mobilisation to plasmids and its further spread to various sised plasmids identified in non-clonally related *K. pneumoniae* and *P. aeruginosa* isolates.

P2011 Diversity of blaOXA-48 genetic environments leading to variable carbapenemase expression levels among carbapenem-resistant *Klebsiella pneumoniae* isolates

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Objectives: The aim of this study was to investigate the mechanisms responsible for variability of resistance to carbapenems among 39 carbapenem-resistant *Klebsiella pneumoniae* isolates expressing the carbapenem-hydrolysing oxacillinase OXA-48 and involved in a hospital outbreak in Istanbul, Turkey.
Methods: Genetic relationship of the 39 *K. pneumoniae* isolates was assessed by pulsed-field gel electrophoresis (PFGE). The genetic environnement of the blaOXA-48 gene was studied by PCR combination using specific primers of IS1999 and blaOXA-48 followed by sequencing. The level of expression of blaOXA-48 was evaluated by hydrolysis assay using culture extracts of blaOXA-48-positive *Escherichia coli* transconjugants.
Results: PFGE analysis revealed two distinct genotypes featuring clones A and B (25 and 14 isolates, respectively). The IS1999 element was identified upstream of the blaOXA-48 gene in both clones, providing promoter sequences for blaOXA-48 expression in clone A isolates. Among isolates belonging to clone B, insertion sequence IS1R was inserted within IS1999 thus forming an hybrid promoter for blaOXA-48 expression. Hydrolysis measurements showed that the level of expression of blaOXA-48 was twice in clone B than in clone A isolates.
Discussion: This study showed that higher resistance to carbapenems in some blaOXA-48-positive *K. pneumoniae* isolates might be related

to higher expression of this carbapenem-hydrolysing oxacillinase gene. This discrepancy was linked to the insertion of an IS1R element forming an hybrid promoter. It indicates an evolution of those OXA-48-positive *K. pneumoniae* isolates disseminated in Istanbul toward higher carbapenem resistance.

P2012 In vivo selection of carbapenem resistance in *Acinetobacter baumannii* mediated by ISAba1 mobilisation upstream of the blaOXA-51 natural oxacillinase gene

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Objectives: To analyse the mechanism(s) involved in the acquired resistance to carbapenems in *A. baumannii* under in vivo imipenem selection pressure.
Methods: Species identification was done by the biochemical API32 GN test (bio-Mérieux, France) and by 16S rRNA sequencing. Susceptibility testing was done by disk diffusion method and Minimum Inhibitory Concentration (MICs) were determined by agar dilution. Extended-spectrum β-lactamase (ESBL) was revealed by performing double-disk synergy test using disks containing ceftazidime or cefepime and ticarcillin-clavulanic acid on Mueller-Hinton agar plates. AmpC cephalosporinase hyperproduction was evaluated by performing ceftazidime susceptibility testing on cloxacillin-containing plates. Genes coding for the carbapenem-hydrolysing class D β-lactamases OXA-23, OXA-40, OXA-58 and OXA-51 subgroups were searched along with the presence of blaTEM-type and blaSHV-type genes. The isolates were analysed for the presence of ISAba1 in the promoter region of the blaOXA-51-like and blaampC genes in order to investigate the role of ISAba1 in blaOXA-51 and blaampC expression. Genotyping was done by pulsed field gel electrophoresis (PFGE) after digestion by ApaI.
Results: An imipenem-susceptible *A. baumannii* (A1) (MIC of 2 mg/L) isolate was recovered from rectal and wound swabs from an ICU patient. After ten days of imipenem treatment, an imipenem-resistant *A. baumannii* isolate (A2) (MIC 8 mg/L) was recovered from an endotracheal aspirate performed in the same patient. Genotyping revealed that both isolates were clonally-related. Both strains harboured blaTEM-1 gene and ISAba1 upstream of the blaampC gene leading to ticarcillin and ceftazidime resistance. Both strains had a naturally-occurring blaOXA-51-like gene with ISAba1 being identified upstream of the blaOXA-51 gene of *A. baumannii* A2, thus leading to overexpression of this carbapenem-hydrolysing oxacillinase.
Conclusion: This is the first report of an in vivo selection of carbapenem-resistance in *A. baumannii*. This acquired resistance trait was related to selection of an ISAba1 mobilisation event leading to blaOXA-51 overexpression.

P2013 ISEcp1-mediated transposition of qnrB5 gene in *Escherichia coli*

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Objectives: Plasmid-mediated quinolone resistance due to Qnr determinants is increasingly reported worldwide in enterobacterial isolates being of the QnrA, QnrB and QnrS types. The qnrA-and qnrB-like genes are often identified in sul1-type integrons (ISCR1-based mobilisation). The aim of this study was (i) to investigate the genetic environment of a qnrB gene identified in an *Escherichia coli* isolate recovered from Colombia, and (ii) to experimentally evaluate the mobility of that structure in *E. coli*.
Methods: Cloning experiments were performed with EcoRI-restricted whole-cell DNA of *E. coli* clinical isolate R4525 and the genetic environment surrounding the qnrB gene was determined by primer walking. The transcription start site was determined by 5'RACE analysis. The ISEcp1-mediated mobilisation of the qnrB5 gene was attempted from *E. coli* R4525 to recA (–) *E. coli* recipient strain using self-conjugative and IS-free plasmid pOX38-Gen as a target for transposition events.
Results: The qnrB5 gene was located at the right-hand extremity of the ISEcp1 element, forming a 2,739-bp long transposon flanked by a 5-bp duplication of the target site (ATCAA) and inserted inside orf1 of Tn1721. As opposed to what has been reported for β-lactamase genes of the CTX-M type, the qnrB5 gene was located in an opposite orientation, relative to the transposase ORF of ISEcp1. Therefore, ISEcp1 did not provide promoter sequences for its expression. ISEcp1B-mediated transposition of qnrB5 into *E. coli* was successful in vitro and was found to occur at a frequency of ca. 10–6 per donor by a mechanism similar to one-ended transposition. The promoter sequences able to enhance the qnrB5 expression were determined, being made of a –35 box (TTGACG) and a –10 box (TACCAT) separated by a 17-bp sequence, with the transcription start site located 30 bp upstream of the codon start. This allowed us to consider the QnrB5 protein to be 214 amino-acid long thus shorter than previously hypothesised.
Conclusion: These findings show that ISEcp1 is at the origin of acquisition of the qnrB5 gene in *E. coli*. ISEcp1-like elements have previously shown to mobilize β-lactamase genes (blaCTX-M) and rmtC genes (resistance to aminoglycosides).

P2014 *Salmonella enterica* serovar Typhimurium isolates from animal origin encoding blaCTX-M1 and harbouring a non-common integron profile related to a *Salmonella* Genomic Island 1 variant

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Objectives: Molecular characterisation of antimicrobial resistance (R) in seven *Salmonella* (S.) Typhimurium isolates showing an ESBLs phenotype and collected from different animals (4 from horse, 2 from cat and 1 from bird) in the same German region during 2003–2006.
Methods: Among the *S. enterica* isolates from animal and food origin (2003–2007) collected in the National Reference Laboratory for *Salmonella* (NRL-Salm) collection (Berlin) showing full or intermediate resistance to ceftiofur (MIC $\geqslant$ 4 mg/L), 7 S. Typhimurium isolates were selected for this work. All of them came from the same German region, but were isolated from different years/animals. Their susceptibility to 17 antimicrobial agents (including the β-lactams ampicillin and ceftiofur, and amoxycillin/clavulanic acid) by broth microdilution, and also for a broader panel of 11 β-lactams by the disc-diffusion method was tested. Molecular methods as PCR amplifications/sequencing, isoelectric focusing, PFGE with XbaI, plasmid profile analysis and Southern-hybridisation were used to characterize the resistance determinants and resistant isolates.
Results: All seven S. Typhimurium isolates were DT104L and shared a common R-pattern: [AMP-AMCI-CEF-CXM-TIC-PRL-EFT-CRO-CTX-CPD]-[CHL-FLO]-GEN-KAN-TET-SUL, with only minor variations in some antimicrobials: [CAZ-ATM]-STR-SPE-TMP-SXT. The R-genotype contained in all cases [blaCTX-M1-blaPSE-1]-floR-tet(G)-sul1-aadB. Other genes as blaTEM-1, strA-strB, aadA1 or sul2 were also present in some isolates. The strains harboured two class 1 integrons with variable regions of 700 bp and aadB genes, and 1200 bp with blaPSE-1. The detection of this characteristic integron profile together with the presence of the genes floR and tetG suggests the presence of a SGI1-variant. The seven S. Typhimurium isolates present the same characteristic XbaI-profile (frequent in DT104 strains), with variations related to the presence of plasmids. Three different plasmid patterns were found, all of them containing the S. Typhimurium virulence plasmid (90 kb) detected by spvC PCR. The blaCTX-M1 genes were located on three different plasmids with sizes between 90–110 kb.
Conclusions: The presence of blaCTX-M1 and the integron profile 700 bp/ aadB, 1200 bp/ blaPSE-1 in combination with a similar XbaI-type in seven Typhimurium isolates from the same region, are indicative for the presence of a clone in the area which could have changed through time and different animal hosts.

P2015 Prevalence of In99 among *Pseudomonas aeruginosa* isolates from a central region of Portugal (Aveiro) since 2003

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Objective: *P. aeruginosa* is an important pathogen responsible for nosocomial infections, mainly among immunocompromised patients. In the last years, several studies describing the occurrence of integrons and their association with the increasing of antibiotic resistance have been largely discussed. The aim of this work was to investigate the incidence and prevalence of integrons in *P. aeruginosa* since 2003, in a central region of Portugal (Hospital Infante D. Pedro, Aveiro).
Methods: *P. aeruginosa* isolates were recovered from patients of different wards admitted to the hospital within a 48 h period. The isolates were identified and antibiotic susceptibilities were determined by the automatic VITEK 2 system and Advanced Expert System (VITEK 2 AES) (BioMérieux, Marcy L'Étoile, France). The detection of integrons was performed by PCR followed by sequencing. All the nucleotide and predicted amino acids sequences were analysed by Clustal W and Blast programmes. The isolates phylogenetic relationship was investigated by rep-PCR.
Results: Class 1 integrons were identified in 18% of the isolates possessing five distinct gene cassette arrays (In0, In51, In99, In103 and InEc682). In99 conferring resistance to aminoglycosides and β-lactams was the only integron detected in isolates from all years of the timeframe study. InEc682, containing the genes encoding dihydrofolate reductase and ORFD, was present only in four isolates collected in 2003. In0 and In51 were identified once in 2005. In103 was just detected in 2007's strains and it possesses the aac(6')-Il, blaVIM-2 and aac(6')-Ib genes. The presence of class 2, class 3 integrons and plasmids was not detected.
Conclusion: This study demonstrates the prevalence of the In99 integron in the Hospital Infante D. Pedro environment along the years. The same integron was previously identified in a pig's isolate suggesting that other ecological niches, as for instance food production, can be strongly involved in the antibiotic resistance genes influx into the clinical environment. Moreover, so far, In99 and In103 were detected only in isolates from the north and central region of Portugal where the exchange of patients between hospitals is recurrent. Furthermore, to our knowledge, this is the first report of InEc682 in *P. aeruginosa* isolates. Class 1 integrons are spread in a considerable fraction of unrelated *P. aeruginosa* isolates in these hospital-admitted patients and can be useful to complement epidemiological studies.

P2016 Occurrence and molecular characterisation of class 1 integrons in *Acinetobacter baumannii* clinical isolates collected from 1995 to 2006 in Portugal

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Acinetobacter baumannii (Ab) is often resistant to multiple antibiotics. A major role in dissemination and evolution in antimicrobial resistance has been attributed to integrons, especially class 1 integrons. The aim of this study was to investigate the prevalence and the role of class 1 integron in mediating antibiotic resistance in Portuguese Ab clinical isolates. The isolates comprised strains from 1995 until 2006. Previously, we have shown the dissemination of clonally related multidrug-resistant isolates (MDR clone) in various hospitals since 1999, that belongs to a sub-lineage of European clone II [1,2]. Thus, representative isolates from five hospitals, collected over time, were included, as well as, all the isolates recovered before 1999 (showing a more susceptible and variable antimicrobial profile). Overall, 147 isolates were screened for the presence of class 1 integrons by PCR with 5′CS and 3′CS primers, with further sequencing of amplicons. The results showed that more then an half (57.3%) of the isolates carried a class 1 integron and 11.7% had more than one integron. Amplicons of ca 550–600, 800, 1000 and 2500 bp were observed. All amplicons from MDR clone carried an integron of 550 bp, coding for an unknown function protein, while only one code for a protein of ferric transport (according BLAST search). The others code for an aadB allele, IMP-5 determinant and an array cassettes of aaC1, orfX, OrfX′ and aadA1, respectively, already described elsewhere. In conclusion, Portuguese Ab isolates have revealed an unexpectedly high genetic stability over this prolonged period of time with limited diversity of gene cassettes, contributing only in part for the resistance observed. Instead, it could reflect an evolution of a clone capable of acquiring other mechanisms of resistance, whose integron structure appear quite stable over time.

Reference(s)

[1] Da Silva GJ et al. (2007) Clin. Microb. Infect. 13:.190–195.
[2] Da Silva GJ et al. (2004) J. Antimicrob. Chemother. 54:.255–258.

P2017 Comparison of class 1 integrons from human and avian *E. coli*

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Objectives: A prospective study for the identification and comparison of class 1 integrons in *E. coli* from three sources: farm poultry, hospitalised and non-hospitalized patients.
Methods: A total of 235 *E. coli* were collected; 80 from hospitalised patients, 90 from non-hospitalized patients with no prior hospitalisation in the preceding 3 months, and 65 from farm poultry. Identification of the isolates was performed by the Wider 1 automated system and susceptibilities to commonly used antimicrobial agents were determined by the Kirby-Bauer and broth microdilution methods. IntI1-specific polymerase chain reaction (PCR), conserved-segment PCR, and DNA sequencing were used to determine the presence, length, and content of integrons. The relatedness among the isolates was examined by pulsed-field gel electrophoresis of the XbaI digests of genomic bacterial DNA.
Results: The rate of integron carriage was 49.2% for *E. coli* derived from farm poultry, 26.2% and 11.1% for isolates recovered from hospitalised and non-hospitalized patients. The length of the integrons' variable regions ranged from 500 to 2700 bp. 96.8% of integron-carrying organisms exhibited resistance to more than two classes of antibiotics (MDR), whereas only 34.8% of non-integron-carrying organisms were MDR ($P < 0.001$). The most prevalent genes were the aad and dfr gene families detected in 71.4% and 65% of sequenced integrons respectively. Identical integrons were detected in *E. coli* of human and animal sources.
Conclusion: A large reservoir of integrons exists in *E. coli* of food-producing animals. The horizontal transfer of class 1 integrons among bacteria of animal and human origins seems to play an important role in the dissemination of antimicrobial resistance.

P2018 Characterisation of an unusual class 1 integron in *Escherichia coli* isolates of animal origin

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Objective: To characterize the gene cassette arrangement inside an unusual class 1 integrons that lack the 3′-conserved region, detected in *Escherichia coli* isolates of animal origin.
Methods: In a prevalence study of class 1 integrons in cotrimoxazole-resistant *E. coli* isolates of animal origin, two strains (Co125 and C1188) harbouring an unusual class 1 integron that lacked the qacED1 and sul1 genes on the integron 3′-conserved region, were detected. The Co125 and C1188 strains were obtained from faecal samples of a pig and a dog, respectively. The antimicrobial resistance phenotypes were determined by disk diffusion method and the presence of antibiotic resistance genes was analysed by PCR, and sequencing. Based on the known integron sequences previously included in GenBank (accession numbers EF051037 and EF113389), a wide variety of primers were designed to determine the genetic structure of their class 1 integrons,

using the PCR and sequencing primer-walking strategy, and the PCR-RFLP analysis with different restriction enzymes.

Results: The *E. coli* Co125 showed a multiple-antibiotic-resistant phenotype (resistant to nalidixic acid, ciprofloxacin, ampicillin, rifampin, tetracycline, chloramphenicol, sulfamethoxazole, trimethoprim, and streptomycin), and the *E. coli* C1188 was resistant to tetracycline, chloramphenicol, sulfamethoxazole, trimethoprim, and streptomycin. The tetA, cmlA, sul3, dfrA12, aadA1 and aadA2 genes were found in both strains, and additionally, the blaTEM1b and dfrA1-like genes were detected in the Co125 strain. The same gene cassette combination associated to the sul3-domain was observed in the unusual integron of both strains, which included in the 7.1 kb sequenced fragment downstream the intI1 gene, the following arrangement: dfrA12-orfF-aadA2-cmlA1-aadA1-qacH-IS440-sul3.

Conclusions: To our knowledge this is the first report of multiresistant unusual class 1 integron structure in *E. coli* isolates of animal origin. The inclusion of a variety of resistance genes inside integrons constitutes an effective means to spread multiple antibiotic resistance among bacteria from different ecosystems.

P2019 Diversity of sul genes (sul1, sul2 and sul3) among human clinical Enterobacteriaceae isolates recovered during the last two decades in Spain (1988–2006)

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Objectives: Enterobacteriaceae are often resistant to antibiotics not currently used in the clinical setting as sulfonamides (sul) or streptomycin (str). The diversity of sul and str genes among extended-spectrum β-lactamase (ESBL) and non-ESBL Enterobacteriaceae is analysed.

Methods: We studied 344 isolates (249 *E. coli*, 56 *K. pneumoniae*, 23 *Enterobacter*, 7 *K. oxytoca*, 6 *Salmonella enterica* Paratyphi, and 3 *Citrobacter*) corresponding to (i) ESBL- and metallo-β-lactamase-producing isolates (TEM-4, -12, -24, -27, -52; SHV-2, -5, -12, -13; CTX-M-1, -3, -9, -10, -14, -15, and -32; VIM-1, n = 234], (ii) clinical non-ESBL isolates (n = 70) and (iii) faecal non-ESBL isolates (n = 40) from 1988 to 2006. Antibiotic susceptibility was tested by disk diffusion (CLSI). Presence of sul and str genes and sequences related to their known genetic platforms (Class 1 integrons, ISCR2, RepCpRSF1010, QacH, IS26) were analysed by PCR and/or hybridisation. Genetic environment was characterised by PCR overlapping, long-PCR-RFLP and sequencing.

Results: Resistance to sulphonamides was similar among ESBL and non-ESBL isolates (55% vs 48%). Sul genes were absent in 6% of sulphonamides resistant and present in 8% of susceptible isolates. They were transferable in 48% of the cases, being mostly associated with streptomycin (79%), tetracycline (77%) and trimethoprim (75%) and sporadically with b-lactam resistance. sul1 was more commonly found than sul2 or sul3 (48%, 36% and 5%, respectively), and presence of more than one sul gene was frequent (27%). sul1 was mainly associated with class 1 integrons (78%) widely distributed in different species and plasmids. sul2 was associated with pRSF1010 sequences (RepC-sul2-strA-strB, 20%), or different ISCR2 platforms (18%): ISCR2-glmM-sul2, ISCR2-strB-strA-sul2 or ISCR2-IS26-strB-strA-sul2. Two strains contained both ISCR2 and RepC-sul2-strA-strB elements. A high number (69%) was not associated with known sequences. sul3 gene was only detected among *E. coli* irrespective of ESBL production (n = 16) and was mostly associated with different unusual class I integrons containing qacH (n = 13).

Conclusions: sul genes are widely spread among human Enterobacteriaceae isolates and sporadically linked to ESBL plasmids. Although most sul genes were associated with widely spread platforms as class 1 integrons (sul1, sul3) or pRSF1010 (sul2), a high number of isolates was not associated with known sequences which indicates other unknown reservoirs for these genes.

P2020 Replicon typing of plasmids carrying the sul3-atypical integrons among *Salmonella* isolates

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Objectives: The spread of atypical sul3-integron platforms containing three different gene cassette arrays has been recently detected among *Salmonella* isolates from Portugal. The aim of this study was to characterize the plasmids associated with these genetic elements in a large collection of Portuguese *Salmonella* isolates.

Methods: Fifty-two representative isolates carrying sul3-atypical integrons (n = 22) and class 1 integrons (n = 30) from different sources (human, food products and environment), serotypes and PFGE-clones were studied. Plasmid analysis included determination of size (Barton's method), comparison of RFLP patterns and characterisation of incompatibility groups by rep-PCR typing method and further hybridisation.

Results: Plasmids belonging to different Inc groups were identified. Those carrying sul3 type I-integrons (5′CS-dfrA12-orfF-aada2-cmlA1-aadA1-qacH-tnpA-sul3-tnp) were diverse and classify as IncI1 (conjugative, 100 Kb, in 2 clones, 2 serotypes), IncN (non-transferable, 135 Kb, n= 1 strain) and FIIA (transferable, 100–165 Kb, 2 clones of 2 serotypes), a common replicon among *Salmonella* virulence plasmids. Plasmids carrying the sul3 type II-integrons (5′CS-dfrA12-orfF-aadA2/1-qacH-tnpA-sul3-tnp), only described in the MDR S. Rissen clone, did not amplify with rep primers we used (70 Kb). The sul3 type III-integrons (5′CS-estX-psp-aadA2-cmlA1-aadA1-qacH-tnpA-sul3-tnp) were located in non-transferable IncA/C plasmids, (150, 170 and 220 Kb) of S. Typhimurium DT104, or in a conjugative IncHI1 plasmid (240 Kb) of a non-DT104 S. Typhimurium isolate. IncFII plasmids were detected in several isolates carrying class 1 integrons, but the majority of these isolates were negative by rep-PCR method.

Conclusion: The atypical sul3-integron platforms are located within plasmids of different Inc plasmid groups, some of them restricted to specific clones, and mostly not related to those carrying class 1 integrons in *Salmonella*.

P2021 Frequency and molecular epidemiology of integron-carrying *Salmonella enterica* ser Typhimurium isolates from the health region of Terres de l'Ebre, northeastern Spain

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Objectives: To compare the frequency and features of class 1 integrons among clinical isolates of *S. enterica* ser Typhimurium recovered over two periods (2000–01 and 2004–06) from patients from Terres de l'Ebre (North East Spain) as well as to investigate the clonal relationship amid isolates displaying identical integron profile (IP).

Methods: Isolates were identified by conventional biochemical methods and serotyped and phagetyped at the National Reference Centre for *Salmonella*. In vitro susceptibility to nitrofurantoin (Nf), amoxicillin (A), tetracycline (T), streptomycin (St), chloramphenicol (C), sulfonamides (Sul), cotrimoxazole (T/S) and other antibiotics was studied by microdilution or disk diffusion. The presence of class 1 integrons and of several resistance genes within their variable region was investigated by PCR; integron gene content from at least one isolate exhibiting each different IP was confirmed by sequencing. Relationship among integron-carrying isolates was assessed by ERIC and REP-PCR.

Results: (a) Class 1 integrons were present in 44.8% (43/96) of isolates recovered during 2000–01 and in 55.4% (51/92) of those recovered during 2004–06. (b) Four different IP were identified [I: 2 Kb/ oxa-1-aadA1; Ib: 2 Kb/ oxa-1-aadA1+ 1.6 Kb/ dfrA1- aadA1; II: 1Kb/ aadA2 + 1.2 Kb/ pse-1; III: 1.2 Kb/ pse-1]. IP I was demonstrated in 20.8% of isolates obtained between 2000 and 2001 and in 35.9% of 2004–06 isolates; IP Ib in 0% and 3.3%; IP II in 24% and 14.1% and IP III in 0% and 2.2%. (c) Resistance phenotypes: IP I [Nf-A-T-St-C-Sul]; IP Ib [Nf-A-T-St-C-Sul-T/S]; IP II [A-T-St-C-Sul] and IP III [A-T-C-Sul]. (d) ERIC and REP-PCR combined patterns revealed six different genotypes

among integron-carrying isolates with most isolates (79%) belonging to a majority genotype, irrespective of their IP or phagetype, while the five minority genotypes were specific of certain IP. Genetic diversity was more common in isolates with IP I.

Conclusions: Frequency of integron-harbouring S. Typhimurium isolates has substantially increased over the research period, at the expense of those carrying an integron bearing oxa-1 and aadA1 genes – which have superseded isolates displaying the IP typical of the pandemic clone of S.Typhimurium DT104. The emergence of integron-related multidrugresistant isolates of S. Typhimurium seems to be mainly due to the spread of a predominant clone, although horizontal transfer of genetic resistance elements may also have occurred.

P2022 Genetic relatedness among isolates of *Shigella sonnei* carrying class 2 integrons in Tehran, Iran, 2002–2003

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Objective: The present study was carried out to investigate genetic relatedness among isolates of *Shigella sonnei* carrying class 2 integrons in Tehran, Iran, 2002–2003.

Material and Methods: Biotyping, drug susceptibility testing, pulsed field gel electrophoresis (PFGE) and analysis of class 2 integrons have been carried out on 60 *S. sonnei* isolates, including 57 sporadic isolates from paediatric cases of shigellosis occurring in 2002 and 2003, two sporadic isolates recovered in 1984 and the ATCC 9290 strain.

Results: Biotype g and resistance to streptomycin, sulfamethoxazole-trimethoprim and tetracycline were exhibited by 54 of the 57 recent isolates. Of the 54 biotype g isolates, 28 exhibited a class 2 integron of 2161 bp, and 24 a class 2 integron of 1371 bp, respectively. Class 2 integrons were not detected in four isolates only, including the two endemic isolates recovered in 1984 and two strains from recent sporadic cases. PFGE divided the strains into eight pulsotypes labeled A to H, three major pulsotypes – A to C – including the large majority of the recent sporadic *S. sonnei* isolates. Pulsotypes A and C were the most prevalent groups, accounting for 41.6% and 35.0%, respectively, of the isolates under study.

Conclusion: The results suggest that biotype g, class 2 integron carrying *S. sonnei* are prevalent in our geographic area. *S. sonnei* isolated in the years 2002 and 2003 could be attributed to a few predominant clusters including, respectively, strains with pulsotypes B and C carrying a 2161 bp class 2 integron, and those having pulsotype A and a 1371 bp class 2 integron. A few epidemic clones are responsible for the apparently endemic occurrence of shigellosis in Tehran, Iran.

P2023 Differences in the distribution of common trimethoprim resistance dfr-genes in *E. coli* and *K. pneumoniae*

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Objectives: The most important dissemination pathway of trimethoprim resistance is through horizontal gene transfer of dfr-genes. Most dfr-genes are carried as gene cassettes in integrons often situated on plasmids. This study describes the distribution of five commonly occurring genes and mobile elements associated with trimethoprim resistance in *Escherichia coli* vs. *Klebsiella pneumoniae*.

Methods: Isolates from urinary cultures of *E. coli* and *K. pneumoniae* resistant to trimethoprim were consecutively collected at the Dept of Clin Microbiology, Växjö during 2004–2007. Within three periods, during 2004, 2005 and 2006, 320 *E. coli* isolates were collected whereas 55 *K. pneumoniae* isolates were collected during the entire period. Susceptibility testing was performed as recommended by the Swedish Reference Group on Antibiotics. Detection of the five most common trimethoprim resistance genes reported in *E. coli*; dfrA1, dfrA5, dfrA7, dfrA12 and dfrA17, was performed using a real-time multiplex PCR. Integrons, class 1 and 2, were screened for with PCR.

Results: The frequency of class I integrons among the *E. coli* isolates was 72% and class II integrons 13%. *K. pneumoniae* isolates carried class I integrons in 38% and class II integrons in 4%.The prevalence of the dfr-genes were in *E. coli* and *K. pneumoniae* respectively dfrA1: 35 and 13%, dfrA5: 16 and 13%, dfrA7: 5 and 2%, dfrA12: 4 and 13% and for dfrA17: 26 and 2%.

Conclusions: The five dfr-genes were found in 86% of the *E. coli* isolates while they only explained trimethoprim resistance in 43% of the *K. pneumoniae* isolates. The dfrA17 gene is one of the most frequent dfr-genes in *E. coli* but was a rare finding in *K. pneumoniae*. On the other hand, dfrA5 was as common in *K. pneumoniae* as in *E. coli*. The integron frequency also differed. This type of descriptive resistance gene data is usually available for the most common species as *E. coli*. As shown here, that information is not always generally applicable to other related species. To define the composition of dfr-genes in *K. pneumoniae*, further characterisation of the material will be performed. The differences observed might be explained by differences in plasmid host range and could be important in understanding the increasing trimethoprim resistance seen in both investigated species.

P2024 Investigation of role of IS elements in conversion to hypermutability and emergence of drug resistance in *Pseudomonas aeruginosa* from CF patients in Cape Town, South Africa

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Objectives: Cystic fibrosis (CF) patients frequently succumb to infections with *P. aeruginosa*, in part due to its ability to adapt to the CF lung environment. Continual antibiotic therapy drives the emergence of multi-drug resistant (MDR) *P. aeruginosa*, further compromising the treatment of CF patients.

A plethora of insertion sequence (IS) elements have been described in *P. aeruginosa*. Some IS elements play a role in emergence of antibiotic resistance through insertional inactivation of genes. Additionally, IS6100 causes large chromosomal inversions (LCIs) that disrupt genes involved in CF lung pathogenesis. IS6100 is of particular concern as it is associated with conversion to hypermutability, resulting in increased rates of spontaneous mutations, often associated with MDR.

The aim of this study was to determine whether IS elements are associated with resistance, particularly to imipenem (IPM), and whether IS6100 plays a role in conversion to hypermutability in *P. aeruginosa* strains from CF patients in Cape Town.

Methods: CF and non-CF *P. aeruginosa* isolates (52) obtained from patients at two local hospitals during 2000–2006 were included in the study.

PCR amplification of oprD from all IPM-resistant strains (75) was carried out to detect insertions that could account for imipenem resistance.

Strains shown to contain IS6100 by PCR were included in Southern hybridisation experiments to determine IS6100 copy number.

Mutator assays were carried out and strains were considered hypermutable if their mutation rates were more than 20 times that of *P. aeruginosa* PAO1.

Results: PCR amplification of oprD in 74 isolates yielded a product of the expected size (1343bp), while the remaining isolate, strain 8, yielded a product of 2400bp. Sequence analysis revealed that oprD from strain 8 was interrupted by insertion of a novel IS element, designated ISPa26. IS6100 was detected in 2 CF and 11 non-CF isolates. Interestingly, strain 6 harbours at least three copies of IS6100, which is associated with LCIs. Of the CF *P. aeruginosa* isolates, 4 were hypermutable. However, isolates containing IS6100 had mutation rates comparable to *P. aeruginosa* PAO1.

Conclusion: Insertional inactivation of oprD by ISPa26 accounts for IPM resistance in strain 8.

As IS6100 was not associated with conversion to hypermutability, further investigation into the genetic environment of IS6100 is necessary as transmission of this element to CF isolates may negatively impact care and treatment of CF patients.

P2025 **The *Acinetobacter baumannii* ATPase gene in diverse strains is frequently occupied by integrated genomic islands**

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Objectives: To determine if the ATPase gene of genetically diverse *A. baumannii* strains harbour integrated genomic islands.
Methods: RAPD-PCR was used to investigate the genetic diversity of fifty *A. baumannii* strains that were originally isolated from various clinical specimens. PCR-based interrogation was used to identify strains with a presumed disrupted ATPase gene. PCR mapping and chromosome walking were then used to provide definitive evidence of the presence of ATPase-borne, chromosomally integrated, genomic islands in these strains. An ATPase-specific yeast capture vector was constructed to enable cloning and characterisation of selected elements.
Results: RAPD-PCR analysis demonstrated that the strains examined possessed substantial genetic diversity. Forty one of fifty strains tested contained a disrupted ATPase gene. Of 15 island-bearing strains subjected to further analysis, 8 were found to contain elements with ends matching one (2 strains) or both (6 strains) extremities of AbaR1, the first *A. baumannii* resistance island to be described; the remaining 7 strains possessed islands with ends distinct to those of AbaR1. Using a recombination-based yeast capture system, we have cloned the integrated element present within the ATPase gene of *A. baumannii* strain A14. In an *E. coli* background, this element confers resistance to ampicillin, chloramphenicol and gentamicin, making it only the second known *A. baumannii* resistance island to be reported.
Conclusions: The ATPase gene of *A. baumannii* strains is frequently occupied by genomic islands. Preliminary data suggest that in addition to AbaR1-related islands, other distinct entities may also be capable of targeting this integration locus.

P2026 **Multiresistance in *Pasteurella multocida* is due to cohabitance of small replicons**

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Objectives: *Pasteurella multocida* is a world-wide-distributed pathogen responsible for a large variety of diseases. In the last years there has been an increase in reports due to *P. multocida* causing human pneumonia and meningitis as a result of pet biting or close contact to farm animals.
Tetracyclines and β-lactams are the two main antimicrobial families used as therapeutic tool against this pathogen. The objective in this work was to study the molecular basis of antimicrobial multiresistance mechanisms in *P. multocida* strains.
Methods: Antimicrobial susceptibility tests were performed following CLSI standard procedures. Pulsed Field Gel Electrophoresis (PFGE) was performed after total DNA digestion with ApaI. A blaROB-1 specific probe was used for Southern Blot. Plasmid Midi Kit (Qiagen) was used for plasmid purification. DNA digestions were performed following manufacturer's instructions. Automated sequencing was carried out at Secugen S. L. (Madrid, Spain). Sequence analysis was performed with DNA Strider software (France).
Results: Out of 604 *P. multocida* animal isolates identified between 2002 and 2005 in our laboratory, thirteen (2.15%) showed high-level resistance to β-lactams. Resistance was due to blaROB-1 borne in plasmid pB1000, a 4613 pb replicon recently described in *Haemophilus parasuis* (San Millan, A et al. Antimicrob Agents Chemother. 2007, 51: 2260–4.). PFGE analysis of the isolates revealed different profiles among the strains, showing that clonal dissemination is not responsible for spread of this phenotype. In addition to β-lactam resistance six of the twelve strains were concomitantly resistant to tetracycline, five were high-level resistant to streptomycin and two were resistant to both molecules. Every single multiresistance pattern from these strains corresponded to a unique plasmid profile, indicating that resistance was related to plasmid content. Complete nucleotide sequence of all plasmids was determined. We describe seven different replicons, each one bearing one, maximum two antimicrobial resistance determinants: pB1000 (blaROB-1), p13142 (tet(O)), p9956 (tet(H)), pTYM1 (sul2, strA), pB1001 (tet(B)), pB1002 (blaROB-1) and pB1003 (sul2, strA). All plasmids could be mobilised into *Escherichia coli*.
Conclusion: Multiresistance in *P. multocida* is due to cohabitance and spread of small plasmids bearing different antimicrobial resistance determinants.

Mechanisms of resistance in Gram-negatives

P2027 **Characterisation of a *P. aeruginosa* mutM mutY double mutant**

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Objectives: The chronic lung infection with *Pseudomonas aeruginosa* in the lungs of patients with cystic fibrosis (CF) is characterised by the biofilm mode of growth and a chronic inflammation dominated by polymorphonuclear leukocytes (PMNs). A high percentage of *P. aeruginosa* strains show high frequencies of mutations (MF) and these isolates also showed significantly higher resistance to anti-pseudomonal drugs. It has previously been shown that hypermutable, resistant *P. aeruginosa* isolates have mutations in genes involved with DNA methyl-directed mismatch repair (MMR).
P. aeruginosa are exposed to many oxygen-radicals both generated by its own metabolism and especially from a huge number of PMNs which release oxygen-radicals in response to the chronic CF-lung infection. To combat the mutagenic consequences of 8-oxodG the mutT, mutY and mutM from the DNA oxidative repair system (GO) plays an important role. Previously it has been found that knock out of both mutY and mutM in *E. coli* have a synergistic effect on the MF.
Methods: We have constructed PA01 mutY mutM double mutant using the Lox system and characterised the mutant through determination of mutation frequency, mutation rate (mutations/cell/generation) and susceptibility using E-test.
Results: Our result demonstrates that in combination this two repair gene enhances mutation frequency over 130 fold compared to PAO1 and 89 and 18 fold increase compared to either mutM or mutY mutator respectively. Mutation rate show similar results. The PA01 mutY mutM double mutant is as strong as the mutS mutator from the MMR system and show a similar susceptibility phenotype with an increase in the size of the resistant mutant subpopulations.
Conclusion: We have found it to be common for clinical CF strains to harbour mutations in several of the repair genes suggesting that interaction of oxygen-radicals and GO deficiency plays a role for the adaptation of *P. aeruginosa* to the stressful CF lung environment. Oxidative stress might be involved in development of resistance to antibiotics and suggest the possible use of antioxidants for prevention of resistance development.

P2028 **Mutagenesis of putative substrate binding residues in the *Escherichia coli* RND type multidrug efflux pump AcrB**

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Background: The *E. coli* AcrB multidrug resistance (MDR) efflux pump confers resistance to a wide variety of chemically unrelated compounds. Recent crystallographic studies including analysis of doxorubicin (DOX)- and minocycline (MIN)-complexed crystals have suggested that the pump is an asymmetric trimer with one protomer forming a pocket rich in aromatic side chains including phenylalanines 136, 178, 610, 615, 617, and 628. To examine whether these residues are involved in the binding/transport of these and other substrates of AcrB, we constructed and tested mutants to identify key residues involved in AcrB multidrug binding.
Methods: A phage lambda based homologous recombination system (Red/ET) was used to introduce an rpsL-neo cassette into acrB of *E. coli* 3-AG100 and to subsequently replace the cassette by an

appropriate oligonucleotide resulting in conversion of phenylalanines into alanine. By the same method we constructed as a control the F628F pseudomutant (wild type amino acid sequence, replacement of TTT for GTT). Susceptibility to various compounds including ethidium bromide (EtBr), Hoechst33342 (HOE), DOX, novobiocin, and the efflux pump inhibitors (EPIs) phenyl-arginine-β-naphthylamide (PAβN) and 1-naphthylmethylpiperazine (NMP) was measured by a microdilution assay. Cellular accumulation of selected substrates was also assayed.
Results: Based on a comparison of the MICs for the F628F pseudomutant and a pump-deficient (AcrB::rpsL-neo) control strain, the "best" substrates (MIC ratio >32) were oxacillin, DOX, novobiocin, macrolides, pyronine Y, and HOE while "intermediate" substrates (MIC ratio 8–32) were linezolid, MIN, EtBr, levofloxacin, ciprofloxacin, propidium iodide, PAβN, and chloramphenicol. Gentamicin, spectinomycin and NMP were no substrates. Mutations F610A and F178A had the most significant impact on substrate MICs. The triple F615A/F617A/F628A mutant affected MIC values of almost all tested substrates. Surprisingly little impact on the MICs were noted for DOX and MIN. Accumulation assay results with DOX, NMP, PAβN, and EtBr correlated with the observed changes in the MICs.
Conclusions: The results are consistent with the view that some of phenylalanine residues 136, 178, 610, 615, 617, and 628 funtion as binding residues of the AcrB protein. The putative pocket formed by these residues provides the flexibility needed for an efficient MDR efflux pump. The EPIs PAβN and NMP may have different primary targets and mechanisms of action.

P2029 Regulation of efflux-mediated multidrug resistance by the ramR-ramA region in *Salmonella enterica* serovar Typhimurium

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Objectives: To study the regulation of efflux system expression in multidrug resistant (MDR) *Salmonella enterica* serovar Typhimurium (S. Typhimurium) DT104 and DT204 strains.
To determine the involvement of two transcriptional regulators in multidrug resistance and in overexpression of the AcrAB efflux pump.
Methods: Identification in the sequenced genome of S. Typhimurium strain LT2 of an open reading frame coding for a putative regulatory protein of the TetR family upstream of the ramA gene whose gene product, homologous to MarA, when overexepressed confers a multidrug resistance (MDR) phenotype in several bacterial species.
Characterisation of the role of this putative regulatory protein gene was done by its inactivation and by complementing experiments. The measure of the expression level of ramA was done by RT-PCR and that of the AcrAB efflux pump by Western blotting. Sequencing of this region was also performed in strains overproducing AcrAB to identify possible mutations involved in deregulation of ramA expression.
Results: Inactivation of the putative regulatory protein gene upstream of ramA in a susceptible S. Typhimurium strain resulted in a MDR phenotype with a 4-fold increase of resistance levels to unrelated antibiotics such as quinolones, fluoroquinolones, phenicols, and tetracycline. Inactivation of this gene resulted also in a 4-fold increased transcription of ramA and a 4-fold increased expression of the AcrAB efflux pump. These results indicated that the gene encodes a local repressor of ramA and was thus named ramR.
In quinolone- or fluoroquinolone-resistant strains of S. Typhimurium overexpressing AcrAB several mutations were identified in ramR which consisted of point mutations resulting in amino acid changes or an in frame shift and also of interruption of ramR by an IS1 element in high-level fluoroquinolone resistant S. Typhimurium DT204 strains. One S. Typhimurium DT104 isolate had a two nucleotide deletion in the putative RamR binding site found upstream of ramA. These mutations were confirmed to play a role in the MDR phenotype by complementing with an intact ramR or inactivation of their respective ramA gene.
Conclusions: The ramR gene coding a transcriptional repressor was identified in S. Typhimurium to control the transcription of ramA. Mutations in ramR appear to play a major role in upregulation of RamA and AcrAB and consecutive efflux-mediated MDR phenotype in S. Typhimurium.

P2030 Mechanisms of amoxicillin resistance in *Haemophilus influenzae* in Spain

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Objectives: To investigate the most important mechanisms of β-lactam resistance in *Haemophilus influenzae*.
Methods: A total of 197 clinical strains of *H. influenzae*, isolated consecutively, were studied. Of them, 55.8% were from children and the majority (90.4%) were isolated from respiratory tract infections. Most isolates were non capsulated, except 1 type f and 2 type b. Amplification and sequencing of the ftsI gene was carried out in all isolates, also, the TEM β-lactamase gene and its promoter were studied in β-lactamase producing strains.
Results: 41.6% of the isolates were β-lactamase-non-producing ampicillin-susceptible (BLNAS), 14.7% were β-lactamase-producing ampicillin-resistant (BLPAR), 34% were β-lactamase-non-producing ampicillin resistant (BLNAR) and 9.6% were β-lactamase-producing amoxicillin/clavulanic acid-resistant (BLPACR). All β-lactamase producing strains were of the TEM-1 type. Of the 48 (24.4%) β-lactamase positive isolates, one (0.5%) contained the P3 promoter, 16 (8.1%) the Pa/Pb promoter, 19 (9.6%) the Pdel promoter, 11 (5.6%) presented the Prpt promoter, and finally, one (0.5%) had a new variant of the Prpt promoter consisting in a double 54 bp insertion (designated 2Prpt). For the Pa/Pb promoter, the geometric mean for amoxicillin was 41.5 mg/l, for the Pdel promoter 44.4 mg/l and for Prpt, 56.4 mg/l. For cefaclor, the geometric mean for the Prpt promoter was 9.66 mg/l, whereas for the Pa/Pb and Pdel promoters, the geometric means were 7.66 mg/l and 7.17 mg/l, respectively.
Conclusions: In the study collection β-lactamase production alone was present in 14.7% of the isolates; 34% had amino acid substitutions in the penicillin-binding protein 3 (PBP3) and both mechanisms were observed in the 9.6% of the isolates. While all β-lactamase positive strains were of the TEM-1 type, a remarkable variation was observed in its promoter region. We describe for the first time, the promoter 2Prpt, which presents an insertion that consists of a double repeat of 54 bp

P2031 Evaluation of active efflux in multidrug-resistant isolates of *Acinetobacter baumannii*

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Objective: The purpose of this study was to evaluate of the contribution of active efflux in intrinsic multidrug resistance in clinical isolates of *A. baumannii* with emphasis on ciprofloxacin, gentamicin, ceftazidime and ethidium bromide (EtBr).
Methods: 65 clinical isolates were obtained throughout a one year period from different clinical samples (urine, blood, bone marrow and burns) from 3 hospitals. Isolates tested were all *A. baumannii*. Using Kirby-bauer drug susceptibility test for 12 antibiotics, 22 multidrug resistant strains which showed resistance to more than 10 of these antibiotics were screened for high resistance to ciprofloxacin, gentamicin, ceftazidime by determining MICs. Subsequently, to show that the high resistance in these strains depend on proton gradient-dependent efflux pump, MICs were also measured in presence and absence of carbonyle cyanide m-chlorophenyl hydrazone (CCCP), using microdilution method. CCCP was added to a concentration of 30 micro M.
Results: MIC for ciprofloxacin, gentamicin and ceftazidime were more than 64, 128 and 256 μg/ml, respectively. CCCP decreased the level of MICs at least in 1 dilution. Consequently the MICs in the presence of CCCP were lower than the MICs in the absence of the reagent.
Conclusion: This study indicates the MICs and accumulation of EtBr in the presence and absence of CCCP were different. These results demonstrate the presence of proton gradient-dependent efflux pumps in clinical isolates of *A. baumannii*.

P2032 **Molecular cloning and functional analysis of a novel ATP-dependent multidrug efflux pump in *Stenotrophomonas maltophilia***

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Objectives: To investigate the presence of a multidrug resistance encoding ABC-type efflux pump in *Stenotrophomonas maltophilia*, a nosocomial pathogen that shows significant degrees of intrinsic and acquired resistance to a wide variety of antimicrobial agents.
Methods: Open reading frames (ORFs) of a hypothetical ABC transporter that are composed of 500 to 800 aa with an N-terminal membrane spanning domain (MSD) and a C-terminal ATP-binding cassette (ABC) domain have been identified in the *S. maltophilia* genome. These were then aligned with experimentally proven antibiotic resistance encoding ABC transporters, and those with significant homology were cloned into a hypersusceptible acrAB mutant *E. coli* strain. MICs of several antimicrobial agents were determined and the post antibiotic effect (PAE) of ciprofloxacin was measured.
Results: The identified ABC transporter (ORF1) has been found to be similar to N-MDR1, C-MDR1, LmrA, and VcaM with amino acid homologies of 39.5%, 41.2%, 42.2%, and 67.5%, respectively. Overexpression of the identified gene led to an 8-fold increase in the MIC of ciprofloxacin, norfloxacin, and tetracycline; other antimicrobial agents have shown a 2-fold increase in the MIC. Heterologous expression of the ABC transporter gene in a supersusceptible *E. coli* strain led to shorter PAE when exposed to ciprofloxacin for 2 hours compared with the control strain expressing only the lacZ gene (0.9 versus 2.5 hours).
Conclusion: ORF1 is a novel ABC transporter in *S. maltophilia*, with a characteristic MSD-ABC domain organisation. Overexpression in a hypersusceptible *E. coli* strain was associated with a significant increase in MICs of several antimicrobial agents including ciprofloxacin, a drug that is commonly used in clinical practice. Additionally, data from the PAE experiment has shown a shorter effect in the strain containing the cloned ABC transporter when compared to its control, suggesting a lower intracellular concentration of the antibiotic in the test strain which may be attributed by a more efficient removal of the antibiotic by the identified ABC transporter.

P2033 **Functional properties of aminoglycoside-resistant methyltransferase Sgm**

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Objective: Aminoglycoside resistance methyltransferase Sgm is a member of the Arm family of enzymes that confer high level resistance to 4,6-disubstituted deoxystreptamines. Recently we have modelled the Sgm structure and analysed the sequence–function relationship by limited proteolysis and mutagenesis. Here we aimed at defining the conditions of methyl transfer, substrate requirements and kinetic parameters of the Sgm enzyme, as well as at its influence on the bacterial cell growth and survival.
Methods: The sgm gene with N-terminal His-tag was cloned into expression vector pET-25b(+), expressed in *Escherichia coli* C41(DE3)pLysS cells and purified to homogeneity. The amount of co-purified cofactor S-adenosyl-methionine (AdoMet) was determined by measuring the A280/A260 ratio. Enzymatic properties of the Sgm enzyme were analysed by methylation test using variable concentrations of Mg(2+) and NH(4)Cl at different pH values. To define the substrate requirements we tried several substrates: entire ribosome, individual subunits and free 23S and 16S rRNA. Kinetic parameters were determined by varying concentrations of cofactor AdoMet and the substrate, while keeping the amount of the enzyme constant. We investigated how the presence of the Sgm enzyme affects the exponential growth of *E. coli* by growing cells in rich and minimal media at different temperatures. In competition growth assays we examined the ability of the cells carrying the Sgm methyltransferase to compete with the cells lacking the enzyme.
Results: Nearly 10% of the purified enzyme contains bound AdoMet. The enzyme is most active in the presence of 10–15 mM Mg(2+) and 90 mM NH(4)Cl at pH 7–8. Small ribosomal subunits are readily methylated by the Sgm enzyme, but neither intact ribosome and 50S subunit or free 23S and 16S rRNA can serve as a substrate. The Sgm follows Michaelis-Menten kinetics with KM for AdoMet of 38 mM and kcat of 0.2 min-1. *E. coli* cells expressing the Sgm grow slightly faster compared with the non expressing strain. The effect is shown both in competition experiments and in individual strain growth.
Conclusions: We defined the enzymatic properties and the substrate of the Sgm enzyme and observed its positive influence on cell growth. Our results set the basis for more detailed studies of the reaction mechanism and recognition elements in 16S rRNA, which will all help to find the specific inhibitor of Arm methyltransferases and thus fight the aminoglycoside resistance.

P2034 **The biological cost of antibiotic resistance in urinary isolates of *Escherichia coli***

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Objectives: The development and stability of a resistant bacterial population is dependent on the biological cost associated with resistance. A previous study showed that fosfomycin resistance in *Escherichia coli* confers a biological cost in vitro. To investigate whether this holds true for other resistance determinants in *E. coli* we have experimentally measured the biological cost of 12 different antibiotic resistance phenotypes in clinical isolates of *E. coli*.
Methods: UTI isolates of *E. coli* resistant to ampicillin, trimethoprim, nalidixic acid and/or mecillinam, alone or in combination (n= 11–54 isolates per phenotype) and *E. coli* susceptible to all ten tested antimicrobial agents, wildtype, (n = 50) were collected at the Dept of Clin Microbiology, Växjö. Susceptibility testing was performed as recommended by the Swedish Reference Group for Antibiotics. Isolates resistant to nitrofurantoin or cephalosporines were excluded. To estimate the biological cost of each phenotype, the growth rate was determined by using BioScreenC, a turbidometric reader which monitors bacterial growth continuously. Each isolate was inoculated to a final density of 10^5 cfu/ml in a well with 0.4 mL LB. Growth was recorded during 8 h at 37ºC as an increase in optical density at 540 nm. Isolates were run in triplicate. Viable count was performed to verify the results for the reference strain (*E. coli* ATCC 25922) and isolates with isolated mecillinam resistance. Student's t-test was used for statistics. $p < 0.001$ was considered significant. Results are presented as means with 95% CI given in parenthesis.
Results: The mean generation times for mecillinam resistant isolates, as single, 26.9 min (25.4–28.5), and as part of a combination with other resistance determinants, 18.7 min (17.2–20.2), were significantly longer than in wildtype isolates, 16.1 min (15.5–16.6). Generation times for other resistance phenotypes did not differ significantly from wildtype.
Conclusions: Based on growth rate measurements in vitro, resistance to ampicillin, trimethoprim and nalidixic acid alone or in combination imposed no measurable fitness cost on clinical isolates of *E. coli*. This implies that once these resistance determinants are established, they are likely to persist despite decreases in selective pressure. In contrast mecillinam resistance conferred significantly reduced fitness. This finding provides a potential explanation to why mecillinam resistance is uncommon among clinical isolates.

P2035 **Analysis of variations in the target gene penA among *Neisseria meningitidis* with decreased susceptibility to penicillin of serogroups other than C: an additional tool to improve typing of meningococcal isolates**

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Objectives: There are many reports correlating decreased susceptibility to penicillin in meningococci (PenI, $0.06 < \text{MIC} < 1$ mg/L) to the mosaicism of the penA gene due to alterations mainly confined in the 3′ region.

In this study, we examined 143 *Neisseria meningitidis* PenI strains isolated from 2002 through the first six months of 2007 to monitor the penA changes among strains with different phenotypes and genotypes and to improve typing of meningococci isolated over time.

Methods: Serogroup and sero/subtype were determined for all the examined strains. Susceptibility to penicillin was evaluated using the E-test method. The breakpoint for penicillin was $\geqslant$0.094 mg/L. The penA gene of the PenI strains was sequenced and each sequence compared with the others and with those deposited in Genbank. The MultiLocusSequenceTyping permitted to assign the isolates to different clonal lineages.

Results: In Italy, the percentage of PenI strains increased from 24% in 2002 to 41% in 2007. In particular, after the emergence in 2002 of the C:2b:P1.5 ST-8 complex/Cluster A4 and in 2004 of the C:2b:P1.5,2 ST-8 complex/Cluster A4, as predominant clones of PenI strains, during the last two years we have observed their gradual decrease in parallel with the increase of serogroup B, W135 and Y Pen I strains belonging to different clonal complexes. The penA sequence analysis showed the presence of 18 variants analysing the entire gene and of 14 variants when considering only an internal 402 bp region corresponding to the most variable part of the gene. Besides the penA12, detected in all strains C2b:P1.5/P1.5,2 ST-8 complex /Cluster A4, the major alleles found were penA14 (mostly among B, ST269), penA9 (mostly found among C, ST11/ET37) and penA20 (exclusively found among Y, ST23/A3).

Conclusion: Invasive disease due to PenI meningococci increased twofold between 2002 and 2007. Molecular analyses defined the different serogroups and clonal lineages that replaced the C2b:P1.5/P1.5,2 ST-8 complex/Cluster A4 during the last two years. They represent the majority of PenI strains recently circulating and are characterised by the presence of specific penA alleles predominant in the current meningococcal population. PenA sequencing could be considered useful not only for identifying isolates with reduced susceptibility to penicillin G but also as an additional target gene for molecular tracing of meningococci over time.

P2036 Genetic basis of florfenicol resistance in *Acinetobacter* isolates from hospital in Chile

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Objective: DNA Sequence analyses suggest that most of the antibiotic resistance genes found in the *Acinetobacter baumannii* strains have been acquired from bacteria of the genera *Pseudomonas*, *Salmonella*, or *Escherichia* via horizontal gene transfer (HGT). A florfenicol resistance gene (floR) was detected in *A. baumannii* strains from two hospitals in Chile. Florfenicol is not approved for human use; however, it is related to chloramphenicol and can select for cross-resistance among bacterial pathogens. The aim of this study was to analyse the floR-flanking regions in *A. baumannii* clinical isolates in order to determine the evolutionary history of the floR gene acquisition in *A. baumannii*, the linkage between floR and other antibiotic resistance genes, and the potential impact that the floR gene acquisition has on human health.

Methods: The genetic regions upstream and downstream of floR were analysed in four floR-positive, phenicol resistant *A. baumannii* strains. These strains were isolated from hospitals in Concepción and Santiago, Chile, between 1991 and 2000. The Universal GenomeWalker Kit (BD Biosciences Clontech) was used to PCR amplify and sequence unknown DNA regions adjacent to the floR gene, according to the manufacturer's instructions.

Results: Comparative sequence analysis of the 10 kb fragment revealed that the flanking regions of the floR gene in all isolates had some similarity to regions within previously described *Klebsiella pneumoniae* plasmid R55, *Escherichia coli* plasmid 10660–1 and *Vibrio cholerae* STX animal isolates. Two open reading frames were detected downstream of floR gene. The deduced amino acid sequence of orf1 is shows homology with putative regulator protein and the amino acid sequence of orf2 to those of a putative transposase.

Conclusion: Flanking regions of these isolates indicate that *A. baumannii* has exchanged genetic material with other bacteria, likely through HGT. The *Acinetobacter* strains sequenced in this project were almost identical in the region flanking the floR gene, even though these isolates were collected in hospitals 500 km apart and over a span of 10 years. Given that *A. baumannii* is a common nosocomial pathogen and the fact that these strains were isolated from hospitals, it is unclear when or where the HGT would have occurred, especially considering that florfenicol is used in animal agriculture and has no use in human medicine.

P2037 Trends in antibiotic susceptibility profile and assessment of tet(O)-mediated tetracycline resistance in *Campylobacter jejuni* isolates from human and poultry sources

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Objective: This study was conducted to determine the trends in *Campylobacter* antibiotic resistance occurring in our setting. Isolates of human and poultry origin were assessed to determine the plasmid profiles and occurrence of the tet(O) gene in *Campylobacter jejuni* strains ciruculating in our population..

Methods: One hundred *C. jejuni* strains of human and poultry origin isolated in 2002–2003 (phase A) and 2005–2006 (phase B) in the Kingdom of Bahrain were evaluated. The disc diffusion and agar dilution methods were used to assess antimicrobial susceptibility and the minimum inhibitory concentrations of erythromycin, ciprofloxacin and tetracycline. Plasmid extraction was performed using QIAprep® Miniprep kit. PCR detection of the tet(O) gene in purified plasma DNA carried out.

Results: Erythromycin resistance rates were low in both chicken and human *C. jejuni* isolates. Ciprofloxacin resistance rates were high with an increasing trend observed in isolates obtained from both sources during Phase B. Tetracycline resistance was higher in chicken (80.9%) compared to human (41.3%) isolates [$p < 0.01$]. However, tetracycline resistance rates in human isolates increased to 60.5% during Phase B. The minimum inhibitory concentration for the tetracycline resistant *C. jejuni* isolates ranged from 16 to >512ug/ml. There were 13 isolates with MIC >512ug/ml with 12 of these being of poultry origin. With the exception of a tetracycline sensitive human isolate, all the remaining 99 isolates harboured plasmids ranging in size from 15–35kb. The most commonly detected plasmids were the 23kb and 35-kb size with significant correlation between tetracycline-resistance and plasmid carriage being observed in isolates of poultry origin. All tetracycline-resistance *C. jejuni* strains were positive for the tet(O) gene, while none of the susceptible strains gave a positive PCR product.

Conclusion: The findings show continued effectiveness of erythromycin for campylobacteriosis but an increasing trend of high ciprofloxacin and tetracycline resistance. Tetracycline resistance is most likely due to transfer of plasmid carrying tet(O) gene between isolates. Possible trafficking of resistance genes between isolates of poultry and human sources has the potential for generating increased resistance in humans even without previous antibiotic exposure.

P2038 Spread of tetracycline resistance genes among German isolates of *Escherichia coli*, *Enterobacter cloacae*, and *Klebsiella pneumoniae* and susceptibility to tigecycline

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Background: Most common mechanisms for resistance (R) to tetracyclines (T) are drug efflux and ribosomal protection. Tigecycline (TGC), a glycylcycline, has been shown to overcome R to T conferred by tet determinants, but R to TGC mediated by multidrug transporters does occur. During a surveillance study (G-TEST) comprising 15 laboratories we screened all bacterial isolates for susceptibility to doxycycline (DOX) and TGC. The objectives of the study were to evaluate the presence

of tet genes among isolates of *E. coli* (ECO), *E. cloacae* (ECL) and *K. pneumoniae* (KPN) and to determine the susceptibility to TGC.
Methods: 718 isolates (300 ECO, 232 ECL, 186 KPN) collected between April and August 2005 were tested. MICs were determined by a central laboratory using the microdilution method according to the German DIN standard. EUCAST breakpoints were applied to interpret MICs of TGC (susceptible ⩽1 mg/L, R > 2 mg/L). 124 isolates with reduced susceptibility to DOX (65 ECO, 30 ECL, 29 KPN) were randomly chosen for the presence of tet(A) to tet(E) and tet(M) using PCR assays.
Results: R to DOX (MIC > 4 mg/L) was more common in ECO (44%) than in ECL (11%) or KPN (27%). 53 of the 65 ECO (82%) encoded for one or two tet determinants and 12 (18%) were negative for all six tet genes. Only tet(A) and tet(B) were detected. A single gene was found in 50/65 (77%) isolates, of which 30 (46%) harboured tet(A) and 20 (31%) tet(B). Three (5%) isolates encoded for both genes. For isolates harbouring tet(A), DOX MICs were 8–16 mg/L in most cases, while the majority of isolates with tet(B) revealed DOX MICs of ⩾32 mg/L. In contrast, nearly all isolates that were negative for the six tet genes had DOX MICs of 2–4 mg/L.
24/30 ECL isolates (80%) and 22/29 KPN isolates (76%) lacked any of the screened tet determinants. Three ECL isolates each (10%) were positive for tet(B) and tet(D). Of the 29 KPL isolates, six encoded for tet(D) (21%) and one for tet(A) (3%).
TGC inhibited all DOX-resistant ECL at 0.5 mg/L, whereas appr. 50% of the DOX-resistant isolates of KPL and ECL displayed TGC MICs above the EUCAST resistance breakpoint of 2 mg/L.
Conclusions: R to DOX in ECO was widespread and related to tet(A) and tet(B) in >80% of the screened cases. TGC was active against all DOX-resistant ECO. In contrast, R to DOX in ECL and KPN, which was often associated with R to TGC, was predominately mediated by other mechanisms of R, probably multidrug transporters.

P2039 Prevalence of nim-genes in *Bacteroides fragilis* group in Belgium

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Objectives: Metronidazole resistance among anaerobic Gram-negative bacilli remains low and below 1 percent in species of the *B. fragilis* group [1] in Belgium. This resistance has been shown to be associated with nitroimidazole (nim) resistance genes. The presence of a nim gene does not necessarily confer metronidazole resistance as nim genes have been detected in *B. fragilis* group isolates that have a MIC below 16 mg/L and are therefore considered susceptible. High level metronidazole resistance can however be induced in nim-gene-carrying *B. fragilis* group strains. The purpose of this study was to determine the prevalence of nim genes in isolates belonging to the *B. fragilis* group isolated in Belgium.
Methods: From October 2003 to February 2005 238 *B. fragilis* group strains were collected in 9 Belgian hospitals [1]. Metronidazole susceptibility was determined by Etest®. The strains were screened for nim genes by PCR with specific primers. When positive, sequencing of the PCR product was performed.
Results: Nim genes were detected in 5/135 (3.7%) of *B. fragilis* strains and 1/103 (0.97%) of other *B. fragilis* group strains. They were not found in isolates with a MIC less than 2 mg/L. Of isolates with MIC higher than 1 mg/L 62.5% (5/8) *B. fragilis* strains and 20% (1/5) other *B. fragilis* group strains were nim gene positive. The only metronidazole resistant *B. fragilis* isolate (MIC=32 mg/L) carried the nimA gene. All other nim-gene carrying *B. fragilis* isolates (2 nimD, 1 nimB and 1 nimE) were susceptible, but with relatively high MIC values (3 with MIC=4 mg/L and 1 with MIC=2 mg/L). In the other *B. fragilis* group strains one B. vulgatus isolate carried the nimD gene and had a MIC of 4 mg/L. The only metronidazole resistant strain (MIC=32 mg/L) in this group was a nim gene negative B. thetaiotaomicron isolate. This either suggests the presence of another resistance mechanism in this strain or the presence of a nim gene, that was not detected with the PCR assay used.
Conclusion: Nitroimidazole (nim) resistance genes were present in 2.5% *B. fragilis* group clinical isolates in Belgium and associated with higher MIC values (range: 2–32 mg/L). The prevalence of nim-genes in *B. fragilis* group clinical isolates in Belgium is comparable with the prevalence other European countries.

Reference(s)

[1] Wybo. I. et al. Third Belgian multicentre survey of antibiotic susceptibility of anaerobic bacteria. Journal of Antimicrobial Chemotherapy 2007; 59: 132–9.

Mechanisms and epidemiology of antimicrobial resistance in Gram-positives

P2040 Tn916-like element EfaB5 genes are highly conserved in invasive *Enterococcus faecalis* and CC17 *Enterococcus faecium*

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Objectives: *Enterococcus faecalis* and *Enterococcus faecium* have evolved in high-risk enterococcal clonal complexes (HiRECC). HiRECC from both species are emerging as nosomial pathogens, most notably the *E. faecium* Clonal Complex 17 (CC17). The extent of virulence gene exchange between both species, however, is unknown.
Methods: We assessed the presence of orthologs in 16 blood culture *E. faecalis* and 97 *E. faecium* of which 40 belonged to the HiRECC CC17, through hybridisation to an *E. faecium* mixed whole genome microarray (Leavis et al. PLoS Pathog 2007). In addition, we performed an in silico hybridisation of *E. faecalis* V583 and the unfinished *E. faecium* DO genome, and interspecies 16s rRNA and MLST genes comparisons. PCR and direct sequencing were used to analyse the spatial arrangement of EfaB5 variant genes in *E. faecium* DO.
Results: *E. faecalis* and *E. faecium* shared only 233 spots (4.4%) in microarray hybridisation of which 58 were >80% specific for CC17 *E. faecium* (sensitivity: 26–98%). Sequencing revealed that eight spots represented genes, present in seven *E. faecalis* strains (n[HiRECC]=5), highly similar to genes previously found on EfaB5, an *E. faecalis* Tn916-like element containing several genes related to virulence in other species. An in silico genome comparison, identified 24 *E. faecium* DO genes, with >85% sequence identity with EfaB5, thus confirming the presence of an element similar to EfaB5 in *E. faecium*. Analysis of spatial arrangements of the EfaB5 orthologues in *E. faecium* DO revealed a conserved 3′ end of this mobile element, while genes with high identity (>95%) to the 5′-end of EfaB5 were found to be scattered on the *E. faecium* DO chromosome. In contrast to the high similarity of EfaB5 genes, the sequence identity of the core genomes of the two species is low, which is reflected by the relative high average of 16S rRNA sequence divergence (4.1%) and the low average nucleotide identity of housekeeping genes (74.2%).
Conclusion: The presence of highly similar genes in CC17 and HiRECC *E. faecalis* suggests genetic exchange between the most prevalent clinical enterococcal subpopulations, in which EfaB5 might have played a role in niche adaptation. Scattered presence of part of the EfaB5 variant genes in *E. faecium* DO, suggests either recombination after integration or acquisition at different occasions.

P2041 Gene expression of vancomycin-sensitive *Staphylococcus aureus* strain EMRSA-15 following exposure to sub-inhibitory levels of vancomycin

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Objectives: Vancomycin is the drug of choice for treatment of infection with MRSA. It is possible that MRSA may be exposed to sub-lethal concentrations of vancomycin during therapy. This study aimed to investigate the effect of sub-lethal concentrations of vancomycin on global gene expression in a vancomycin sensitive strain of EMRSA-15.

Methods: A vancomycin sensitive strain of EMRSA-15 was grown in Brain Heart Infusion broth with and without 0.25 mg/l (sub-inhibitory level) of vancomycin. mRNA was extracted from the two samples using Qiagen RNeasy Kits with RNA stabilisation reagent and 'on-column' DNase treatment. Integrity of RNA was checked by electrophoresis in agarose gels and the quantity of RNA recovered was determined using a Bioanalyser. Investigations were performed in three biological replicates. Gene expression was compared for the isolates using microarray slides spotted with PCR products representing every gene from seven *S. aureus* sequenced genomes. RNA probes were labelled using Cy3 and each prep was hybridised against a common reference of Cy5 labelled genomic DNA extracted from strain EMRSA-15. The slides were scanned by using the Microarray Core Facility at the University of Manchester using a Genepix 4000B scanner (Axon instruments, UK). BlueFuse for microarray 3.3 (BlueGnome, UK) was used to convert all scanned images to raw data. Data analysis was performed in GeneSpring 7.3 (Silicon Genetics).
Results: Gene expression data were analysed using a median normalisation strategy and significant differences in gene expression were identified using ANOVA with Benjamini and Hochberg false discovery rate correction. Comparison of gene expression in the EMRSA-15 strain grown with and without sub-inhibitory levels of vancomycin showed significant differences. Many of the genes with altered expression were involved in cell wall biosynthesis and regulation, but of particular interest was the up regulation of genes in the locus responsible for capsule synthesis.
Conclusion: The data presented support the suggestion that exposure to vancomycin can influence gene expression in MRSA and that growth at sub-lethal concentrations may drive the development of resistance through physiological modifications.

P2042 IS257 and heterogeneous location of ileS2 gene in high-level mupirocin resistance staphylococcal plasmids

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Objectives: High-level mupirocin resistance (Hi-Mup^r) in *S. aureus* is associated with an additional isoleucyl-tRNA synthetase that is encoded by the ileS2 gene. We have previously reported that the ileS2 was carried on 9 conjugative plasmids belonging to 4 structural groups (i.e. S1 to S4) and that the location of ileS2 varied between groups. Different sizes of ileS2-hybridising HindIII plasmid fragments permitted 5 polymorphs of the ileS2 locus to be distinguished. In pUSA03 and pGO400 Hi-Mup^r plasmids the ileS2 gene has been found to be flanked by 2 directly repeated copies of IS257. In this work we analysed the basis of the heterogeneous location of the ileS2 gene.
Methods: The study attempted to detect the existence of IS257 flanking the ileS2 gene, and to amplify the spacer regions between the upstream (IS-L) and downstream (IS-R) copies of IS257 and ileS2 from each of the 9 Hi-Mup^r plasmids. Spacer regions confirmation was done by hybridisation with 2 probes for the ileS2 and an IS257 internal probe.
Results: IS-L and IS-R copies of IS257 were present in all 9 Hi-Mup^r plasmids. The copy of the IS-L was detected in either orientation and the sizes of the spacer regions (with IS257 in either orientation) vary. Heterogeneity was also observed downstream to ileS2. Plasmids of the same group showed an identical organisation of IS257-ileS2 spacer regions. The IS-L copies in plasmids belonging to S1 and S2 groups (i.e. ISpS1-L, ISpS2-L) are in the same orientation as ileS2. On the other hand, in S3 and S4 plasmids the IS-L copies (i.e. ISpS3-L, ISpS4-L) are in the reverse orientation. ISpS1-R, ISpS2-R and ISpS3-R are in the same orientation as ileS2, contrary to the case of ISpS4-R. Considering both sides, different groups have distinct IS257-ileS2 organisations. As in pUSA03 and pGO400, plasmids belonging to S1 and S2 groups showed directly repeated copies of IS257 but upstream IS257-ileS2 amplifications differed in size. S3 group plasmids have inverted copies of IS257, while in plasmids belonging to S4 group, both copies were in opposite orientation to ileS2.
Conclusion: The results support the hypothesis that IS257-mediated events are responsible for the variable location of the ileS2 gene in Hi-Mup^r plasmids. The IS257-ileS2 spacer regions heterogeneity could potentially be used as an epidemiological tool for monitoring Hi-Mup^r plasmid dissemination. The molecular basis for the heterogeneity observed requires additional detailed characterisation.

P2043 Increased mutation frequency among *Enterococcus faecium* belonging to clonal complex 17

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Objectives: Mutator phenotype might confer a selective advantage since different studies have demonstrated a link between elevated mutation frequencies (mutator phenotype), antibiotic resistance and pathogenicity. The aim of this study is to assess whether the distinct genetic *Enterococcus faecium* Clonal Complex 17 (CC17) subpopulation, responsible for the majority of *E. faecium* nosocomial infections and characterised by high antibiotic resistance and recombination levels, presents a high mutation frequency (MF) compared to non-CC17.
Methods: 46 epidemiological unrelated *E. faecium* strains (22 CC17 and 24 non-CC17 strains) were included. MF was determined by calculating the proportion of CFU on Mueller-Hinton agar (MHA) supplemented with 256 mg/L of fosfomycin plus 100 mg/L of glucose-6-phosphate (after 48 h of incubation) versus total number of viable cells on MHA without supplements. All strains were susceptible (MIC range, 16–64 mg/L) to this concentration of fosfomycin at the start of the experiment. Experiments were performed in triplicate for each strain and mean values were calculated. Stability of mutants was assayed in selective plates with fosfomycin. The Mann-Whitney U test was used to determine the statistical significance of MF differences between CC17 and non-CC17 ($p < 0.05$ was considered statistically significant).
Results: Among 46 *E. faecium* strains MF ranged from 7×10^8 to 5.2×10^6 (median 6.3×10^7). Stability was demonstrated in all fosfomycin mutants (5 colonies from each plate). The MF of the 22 CC17 strains ranged from 1.3×10^7 to 5.2×10^6 with a median of 1.5×10^6 and was significantly higher than that of the 24 non-CC17 strains ranged from 7×10^8 to 3.1×10^6 with a median 3.7×10^7 ($p = 0.001$). CC17 strains with an elevated MF ($>1\times10^6$) were not confined to particular STs but were randomly distributed among different STs or sublineages within CC17.
Conclusions: This study shows for the first time that strains belonging to the *E. faecium* CC17 genetic subpopulation have an increased MF. This increased MF may have promoted the emergence of antibiotic resistance, most notably ampicillin resistance, within this subpopulation providing a selective advantage that facilitated subsequent adaptation to the hospital environment.

P2044 Efflux-mediated response of *Staphylococcus aureus* exposed to ethidium bromide

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Objectives: Although over 10 efflux pumps (EPs) are described for *S. aureus*, their specific role in drug-response is scarcely characterised. Adapting *S. aureus* to increasing concentrations of a known substrate of EPs, ethidium bromide, in order to increase EPs intrinsic activity and characterising phenotypically and genotypically the resulting progeny, we were able to characterize the molecular mechanisms of adaptation of *S. aureus* to an EP substrate.
Methods: *S. aureus* ATCC25923 was grown in increasing, 2-fold concentrations of ethidium bromide (EB), originating ATCC25923-EB. The MICs of 8 classes of antibiotics, 8 biocides and 2 dyes against ATCC25923 and ATCC25923-EB were determined by broth microdilution (CLSI), with/without efflux pumps inhibitors (EPIs) chlorpromazine (CPZ), thioridazine (TZ), reserpine (RES), verapamil (VER), ouabain (OUA) and carbonyl cyanide m-chlorophenylhydrazone (CCCP). Efflux activity, in EPIs presence/absence, was evaluated by

a semi-automated real-time fluorometric method. The presence and expression of 8 EP genes were assayed by PCR and RT-PCR, respectively. Mutations in both grlA and gyrA quinolone resistance coding regions (QRDR), as well as in the norA promoter region, were assayed by DNA sequencing.

Results: Compared to its parental strain, ATCC25923-EB had a 32-times higher MIC for EB (6.25 to 200 mg/L), as well as decreased susceptibility to biocides and hydrophilic fluoroquinolones; all of which could be reduced by the EPIs TZ, CPZ and RES. Whereas ATCC25923 showed no detectable efflux activity, ATCC25923-EB had pronounced efflux activity which was inhibited by TZ, CPZ and RES in a concentration-dependent manner. Both ATCC25923 and ATCC25923-EB carried norA, norB, norC, mdeA, mepA and sepA, whereas the genes for EPs QacA and Smr were not detected. RT-PCR showed that norA is 35-fold over-expressed in the ATCC25923-EB culture, whereas the remaining EP genes showed no significant increase in their expression. Sequencing of the norA promoter region detected a 70 bp deletion in ATCC25923-EB. No mutations were detected in grlA and gyrA QRDR regions.

Conclusion: Exposure of *S. aureus* strains to quaternary compounds such as EB results in a decreased susceptibility of the organism towards a wide variety of compounds, including quinolones, biocides and dyes through an efflux-mediated response, which for strain ATCC25923, is mainly NorA-mediated. This altered expression may result from alterations in norA promoter region.

P2045 Selection of *Staphylococcus aureus* resistant to NXL101 and identification of associated mutations in gyrA

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Objective: NXL101 is one of a new class of antibacterial gyrase and topoisomerase IV inhibitors, showing potent activity against Gram-positive bacteria including meticillin, fluoroquinolone, vancomycin and macrolide resistant strains. The aim of this study was to evaluate the potential of selecting for resistance to NXL101 and to characterize the associated mutations in the QRDR regions of gyrA, gyrB, grlA and grlB in *S. aureus* clinical strains.

Method: Using a modified method of Szybalski, a geometric gradient of NXL101 concentrations from 0.03 to 32 mg/L, was created in a square Petri dish. 49 *S. aureus* strains (7 strains for which the complete genome is known, and 42 clinical strains exhibiting various meticillin-susceptible (S) or -resistant (R) and fluoroquinolone-S or -R phenotypes) were tested for acquisition of resistance to NXL101, using 6 different inocula for each strain, from 10^5 to 10^{11} CFU/ml. All strains were originally NXL101-susceptible (MIC 0.03–0.125 mg/L). NXL101-resistant clones were collected after incubation at 24 h at 35ºC, their associated MIC values verified and QRDR regions from 1 clone of each resistant strain sequenced.

Results: A significant decrease of susceptibility to NXL101 (MIC $\geqslant$8) was only observed at high inoculum (out of the 49 strains, 5 gave rise to a MIC of 8–32 mg/L at 10^{10} CFU/ml and 21 to a MIC of 8–64 mg/L at 10^{11} CFU/ml).

Sequencing of the QRDR regions of the 21 resistant strains obtained at 10^{11} CFU/ml showed that resistance to NXL101 was associated with mutation in the QRDR region of gyrA only. The mutations are different from those known to be associated with fluoroquinolone-R. The susceptibility profile of these strains to fluoroquinolones remained unchanged after selection for resistance to NXL101.

Conclusion: Resistance to NXL101 was obtained at high inoculum only, suggesting a low spontaneous mutation frequency in response to this compound.

The mutations associated with NXL101-R were different from those associated with fluoroquinolone-R, which is consistent with the lack of cross-resistance observed between NXL101 and fluoroquinolones.

P2046 Induction of telithromycin resistance by several macrolide antibiotics in *Enterococcus* spp. and *Streptococcus* spp.

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Objectives: Macrolide resistance in *Enterococcus* spp. and *Streptococcus* spp. is an increasing problem worldwide. Telithromycin (TL) was developed as an alternative for the treatment of respiratory diseases caused by microorganisms resistant to macrolides. We evaluated the resistance to this antibiotic in 10 strains belonging to both genera, all of them resistant to macrolides and with differences in their genotypic and phenotypic characteristics.

Methods: We selected 10 clinical isolates resistant to erythromycin (ER) collected at our Hospital. All isolates showed flattening of the inhibition halo of TL induced by several macrolides by the disk diffusion method. The isolates selected for this study were: 4 *Enterococcus* spp. (1 *E. casseliflavus*, 1 *E. avium*, 1 *E. faecalis* and 1 *E. faecium*) and 6 *Streptococcus* spp. (2 *S. agalactiae*, 2 *S. pyogenes*, 1 group G *Streptococcus* and 1 group F). We determined their phenotypic patterns by disk diffusion test and their genotypes by PCR amplification with specific primers. Susceptibility to different macrolides was determined by the agar dilution method. MICs of TL were also analysed after induction with several MLS antibiotics.

Results: All strains were resistant to ER and azithromycin with MIC90 > 256 mg/L and 128 mg/L respectively. Only three isolates were resistant to TL. Four strains harboured erm(B) gene, one strain erm(A) gene, and one strain mef(A) gene. We observed reduction of the inhibition halo of TL after induction with macrolides in 4 of the microorganisms analysed (1 *E. avium*, 1 *E. faecalis*, 1 group F and 1 group G *Streptococcus*). However, only in the group F *Streptococcus*, an increase in MICs of TL values was observed.

Conclusions: 1) Resistance induction to TL by macrolides is common in all species of these genera, and it is independent of genotypic and phenotypic characteristics. 2) ER is the higher inductor of TL resistance of all the macrolides studied. 3) Absence of correlation between increased MICs values and reduction of the inhibition halo of TL is probably due to differences in sensitivity of the methods.

P2047 Characterisation of the mef gene in clinical isolates of *Streptococcus pneumoniae* collected during 2004–2005

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Objectives: To determine the presence of a chimeric variant of the macrolide efflux gene mef in *Streptococcus pneumoniae*, consisting of mef(A) between 1–570bp and mef(E) from 619bp to the end, previously found in *Streptococcus pyogenes*, isolated from the Far East.

Methods: Isolates of *S. pneumoniae* collected from the Far East with MIC values demonstrating the M phenotype of resistance to macrolides were tested for the presence of the mef gene. A random selection of 85 of these isolates were sequenced using primers producing a 920bp region of the 1218bp mef gene.

Results: One (1) isolate had 100% homology to the *S. pneumoniae* mef(A) gene published in genbank (accession number AF227520). When compared with the mef(E) gene (accession number U83667), 1 isolate had a single nucleotide polymorphism (SNP) resulting in a stop codon at position Q147. Two (2) isolates had a SNP resulting in a stop codon at position E248. All other isolates were 100% homologous to the mef(E) gene.

Conclusions: The chimeric variant of mef was not identified in *S. pneumoniae* collected 2004–2005 from the Far East. The degree of variation of the mef gene in *S. pneumoniae* is considerably less than the variation of the mef gene in *S. pyogenes*. Interestingly, 3 isolates had a stop codon within the coding region of mef. Further investigations are required to identify whether these isolates are expressing a functional gene or whether the M phenotype of macrolide resistance is being caused by another resistance mechanism.

P2048 Emergence of a linezolid-resistant *Staphylococcus cohnii* after exposure to this drug

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Objectives: This report describes the first linezolid resistant *Staphylococcus cohnii*, recovered from blood culture in a patient after exposure to the agent.
Methods: A 84 year old female patient was admitted to the ICU because of necrotising pancreatitis. She was treated with broad spectrum antimicrobial agents as well as antifungals, because of VAP and severe sepsis due to multidrug resistant Gram-negative bacteria and/or to *Candida glabrata*. The patient was exposed twice to linezolid in normal doses, primarily for eight days (treating Vancomycin Sensitive Enterococci-VSE) and secondly for twelve days (treating VRE). After the second exposure to linezolid, a *Staphylococcus* spp. was isolated from blood culture. Identification of the isolate to the species level and susceptibility testing was done using an semi-automated system (Wider I Fransisco Soria Melguizo, S.A). In order to verify its linezolid-resistance, MIC was re-determined by E-test, while the presence of G2576T mutation was detected by amplification of 23S rRNA following NheI restriction.
Results: The isolate was identified as *Staphylococcus cohnii* and expressed resistance to meticillin (MIC: 16 mg/l), gentamicin (MIC: 16 mg/l), levofloxacin (MIC: 8 mg/l), clindamycin (MIC: 8 mg/l) and linezolid. The MIC to linezolid was 24 mg/l by both methods, while the strain carried three out of four mutated G2576T alleles, responsible for the expression of linezolid-resistance.
Conclusion: Exposure to linezolid enhances the G2576T mutations of 23S rRNA, resulting in treatment failure.

P2049 The epidemiology of invasive group A streptococcal infection in the North American Arctic, 2000–2006

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Background Invasive group A streptococcus (GAS) infection causes morbidity and mortality in the Arctic; however, the epidemiology of this disease is not well described in this region.
Methods: We reviewed population-based data on invasive GAS disease in Alaska (AK) and Northern Canada (N Can) collected from 2000–2006 through the International Circumpolar Surveillance (ICS) network. We defined invasive GAS as an isolate from a normally sterile site (or surgical wound/deep soft tissue infection) from a surveillance region resident. Isolates were forwarded to reference labs in AK and N Can for confirmation. In AK and N Can, emm typing and M typing were performed, respectively. Chart reviews were conducted on lab-confirmed cases.
Results: During the study period, a total of 277 cases of invasive GAS disease were reported from AK (214) and N Can (63); 155 (56%) occurred in indigenous persons. Median age was 42 years, 54.5% were male; 29 (10.5%) cases were fatal. Common clinical presentations included cellulitis (32%), pneumonia (14%), and bacteraemia of unknown source (21%). 11 (4%) cases of necrotising fasciitis occurred; the case fatality ratio for necrotising fasciitis was 27%. In AK, 163 (76%) GAS isolates were available for emm typing; 30 different types were identified; the 3 most common were emm type 3.1 (10%), 41.2 (9%), and 12 (8%). In N Can, 48 (76%) GAS isolates were available for M typing; 17 different types were identified; the 3 most common were M type 3 (12%), 1 (12%), and 5 (10%); 10 (20%) N Can isolates were M non-typable. There was no association between M/emm type and disease presentation, including necrotising fasciitis. In AK, annualised incidence rates were 11.5 and 3.0 cases per 100,000 persons among indigenous and non-indigenous persons, respectively (RR = 3.8, $p < 0.01$). In N Can, rates were 10.2 and 1.1, among indigenous and non-indigenous persons, respectively (RR = 9.3, $p < 0.01$). In N Can, incidence rates of invasive GAS rose over the study period from 5.2 cases in 2000 to 13.5 cases in 2006 ($p = 0.04$). The increase GAS disease incidence was not associated with proliferation of a single M/emm type. In AK, annualised incidence rates demonstrated no discernable trends over the study period.
Conclusions: GAS remains an important cause of invasive bacterial disease in the North American Arctic. Rates of invasive disease with GAS were higher among indigenous persons. Incidence rates in N Can appear to be rising, which merits further evaluation.

P2050 Effect of upstream regulatory regions and growth conditions on erm(B)-mediated constitutive MLS resistance in Group A Streptococci

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Objective: The upstream sequence of the gene is considered a key factor in the translational control of erm expression, which can lead to either a constitutive (cMLS) or an inducible (iMLS) macrolide, lincosamide, and streptogramin B resistance phenotype. We observed that some GAS expressing a cMLS phenotype and harbouring erm(B) did not show a constant phenotype. The present work sought to study the relationship occurring between this phenotypic instability and the sequence context of the erm(B).
Methods: cMLS phenotype of five GAS clinical isolates was studied by growth in liquid medium using cells induced by preincubation with ERY (0.1 mg/L) followed by challenge with either josamycin (JOS) or clindamycin (CLI) (both at 50 mg/L) in supplemented Muller-Hinton II (MH). All cultures were incubated at 35–37ºC in 5% CO_2 for a maximum of 24 h. The effect of inoculum preparation (broth culture or direct colony suspension) was also evaluated.
The sequence of the erm(B) regulatory regions were determined following standard procedures and the general characteristics of the genetic elements harbouring erm(B) were determined using PCR.
Results: When inoculum was prepared by direct colony suspension, both CLI and JOS resistance of 3 strains was induced during the logarithmic phase of growth ((i-cMLS). When the inoculum was prepared by the growth method, the same set of strains were insensible to the induction of CLI and JOS resistance but were susceptible to both antibiotics until the end of the logarithmic phase.
In these strains, the upstream regulatory sequences and the genetic context surrounding erm(B) were identical. A fourth strain had the same genetic characteristics of the i-cMLS strains but showed a constitutive MLS resistance in all the tested growth conditions, as well as a cMLS strain used as reference, the latter having a consistent deletion of the upstream regulatory region of the erm(B).
Conclusions: In GAS, the expression of erm(B) mediated MLS resistance strongly depends on growth conditions. The contribution of erm(B) specific sequences seems to play a secondary role.

P2051 Contribution of drinking water for the dissemination of Gram-positive and Gram-negative bacteria harbouring antibiotic resistance genes in Portugal

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Objectives: To assess the contribution of nontreated water for human use in the spread of Gram-positive (GP) and Gram-negative (GN) bacteria harbouring antibiotic (AB) resistance or virulence genes.
Methods: Drinking water samples [mines, wells, springs from residential, forest or agricultural areas (n = 5); springs from not specified areas (n = 7)] were collected (North/Centre-2006) and, after enrichment, plated on Slanetz-Bartley and MacConkey agar with/without AB. AB susceptibility was done by disk diffusion method (CLSI) and ESBL expression (in GN) was searched by double disk synergy test. Species were identified by API ID32GN (in GN) or PCR (in GP). Characterisation of AB resistance genes/genetic elements, virulence factors (in GP) or *E. coli* phylogenetic groups was done by PCR.

Results: We identified 42 enterococci (7 *E. faecalis*, 1 *E. faecium*, 6 *E. hirae*, 28 *Enterococcus* sp) and 33 Gram-negative (18 Enterobacteriaceae – 1 *E. coli* belonged to phylogroup A; 1 *P. mendocina*, 8 *S. maltophilia*, 2 *A. baumannii*, 1 *A. hydrophila*, 1 *V. parahaemolyticus*, 2 *C. violaceum*). Decreased susceptibility to AB was seen in all sample types to ciprofloxacin-69%, quinupristin-dalfopristin-48%, erythromycin-45%, tetracycline-38%, minocycline-36%, nitrofurantoin-33%, high level of resistance (HLR) to gentamicin-21%, HLR-streptomycin-5% or chloramphenicol-2% in enterococci, and to streptomycin-58%, kanamycin-36%, trimethoprim-27%, tetracyclines-18%, chloramphenicol-18% or sulfonamides-12% in Gram-negative bacteria. The following resistance genes/virulence factors were seen: ermB (31% of all isolates), tetM (40%), tetL (17%), tetS (7%), aac(6′)-Ie-aph(2″)-Ia (21%), asa1 (24%), gel (11%) in enterococci, and class 1 integrons variable regions (6%), sul3 (3%), tetA (3%), and blaTEM-1 (6%) in Gram-negative isolates. Although 2 Enterobacteriaceae were DDST(+) no blaTEM/SHV/CTX-M were seen. We could not amplify vanA/B/C1/C2, tetO/K, cyl/esp/hyl genes in GP and intI1/2/3, sul1/2, qnrA, tetB/G in GN.
Conclusion: Our data suggest that AB resistance genes or putative virulence factors known to be spread in several environments are present in human drinking water both in areas possibly contaminated by humans and in locations that apparently do not have antibiotic selective pressure. The presence of different AB resistance genes/genetic structures is of concern since such bacteria might be consumed by different hosts and/or be involved in gene capture, enriching local environment metagenome.

Epidemiology of antimicrobial resistance around the world

P2052 Susceptibilities to amphotericin B and fluconazole of *Candida* species in TSARY 2006

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Objective: The purposes of this study are two folds. First is to determine the susceptibilities to amphotericin B and fluconazole of the 964 *Candida* species collected in Taiwan Surveillance of Antimicrobial Resistance of Yeasts (TSARY) in 2006. Second is to compare the outcome with the result from TSARY 2002 to determine the trend of susceptibilities.
Methods: The susceptibilities of the isolates were evaluated by the broth microdilution method and the minimum inhibitory concentrations (MICs) of each isolates were determined.
Results: Among the 964 isolates, there were 419 (43.5%) *Candida albicans*, 246 (25.5%) *Candida tropicalis*, 211 (21.9%) *Candida glabrata*, 62 (6.4%) *Candida parapsilosis*, 14 (1.5%) *Candida krusei*, and 12 (1.2%) others. The resistant rate to amphotericin B has decreased comparing to the result from TSARY 2002 (1.8% vs. 2.5%). Among the 17 resistant isolates, 16 were non-*albicans Candida* species, including 12 *C. tropicalis*, 2 *C. krusei*, and one for each of *C. glabrata* and *C. curvata*. On the other hand, there were 165 (17.1%) isolates with MICs to fluconazole ⩾64 mg/l. They consisted of 132 *C. tropicalis*, 14 *C. krusei*, 10 *C. albicans*, and 9 *C. glabrata*. The prevalence of isolates with such high MICs has significantly increased from 1.9% in 2002 to 17.1% in 2006. Among 132 *C. tropicalis* isolates having MICs ⩾64 mg/l, 96 also exhibited trailing phenomenon. Excluding them, there were still 36 (accounting for 14.6% of the 246 *C. tropicalis* isolates tested) considered to be truly resistant.
Conclusions:

1. The resistant rate to amphotericin B has decreased comparing to the result from TSARY 2002 (1.8% vs. 2.5%).
2. The prevalence of isolates with MICs to fluconazole ⩾64 mg/l has significantly increased from the 1.9% in 2002 to the 17.1% in 2006.
3. High prevalence of *C. tropicalis* with MICs to fluconazole ⩾64 mg/l is an emerging problem in Taiwan.

P2053 Is trimethoprim adequate as first-line empirical treatment for urinary tract infection?

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Objectives: To establish whether bacterial urine culture isolates at Mid Staffordshire General Hospital are susceptible to our 1st {Trimethoprim(TMP) or Nitrofurantoin(NIT)} and 2nd {Ciprofloxacin(CIP), Cephalexin(CEP), Augmentin(AGM)} line empirical antibiotic policy.
Methods: During a 5-year period, all uropathogens and the antibiotic sensitivities, causing UTI were identified through our electronic database and analysed, retrospectively. Patients demographics, age, sex, date of urine culture isolate and antibiotic sensitivities were recorded. Data were analysed using SPSS® version 13 for Windows® (SPSS, Chicago, Illinois, USA).
For tables in which both rows and columns contain ordered values, Spearman's rank correlation coefficient, rho, was used. Only Mid Stream Urine cultures with single uropathogen growth of >10^3 CFU/ml and leucocytes of >30cu/ml were included.
Results: The mean age of the population considered was 59 years and the standard deviation was 24 years. UTI was diagnosed more frequently in women (80%). E. Coli was the most common uropathogen isolated and considered responsible for 59.4% of the UTIs. There were 40.770 uropathogens isolated, of which 72.5% were susceptible to TMP, 90% to NIT, 94% to AGM and CIP and 86% to CEP.. A general trend of increasing resistance to elder patients (63% sensitivity for patients >85 years old, rho spearman = 0.00) and in the more recent years, (70% sensitivity for 2007, rho spearman = 0.00) for TMP, was noted. NIT had a resistance rate of 10% for 2007. The 2nd line antibiotics have reduced their susceptibility over the years, but still maintain a susceptibility of about 90% for AGM and CIP and 85% for CEP. Specifically, CEP and NIT still uphold their efficacy, with a sensitivity of 90% for NIT and 85% for CEP, which remained stable over the 5-year period, with statistically insignificant change of susceptibility over the five years (rho spearman = 0.97 for NIT and rho = 0.065 for CEP).
Conclusion: Resistance to TMP is becoming problematic to several geographic areas of UK. In view of increasing resistance pattern to TMP a re-modelling of the local antibiotic policies was considered to be appropriate. Staffordshire hospitals looked into changing the local guidelines to meet new resistance antibiotic patterns, with the use as 1st line antibiotic for empirical treatment of UTI of NIT or CEP and for 2nd line the use of CIP or AGM, excluding TMP from empirical treatment of UTI.

P2054 Resistance among isolates of *Haemophilus influenzae* to orally administered β-lactams and fluoroquinolones: results of a nationwide surveillance study in Germany, winter 2007

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Background: *Haemophilus influenzae* (HI) is an important pathogen responsible for community-acquired infections of the ear, nose and throat (CA-ENTI) and of the lower respiratory tract (CA-LRTI). The main mechanism of resistance (R) to β-lactams (BL) in HI has been considered the production of β-lactamase (BLA), but BLA-negative amino-penicillin-resistant strains, which often exhibit reduced susceptibility to cephalosporins, have also become a problem in some areas. In contrast to BL, R to fluoroquinolones (FQ) is rare in HI, but dissemination of a FQ-resistant clone has been observed (AAC 2004;48:3570–2). Here we report the current resistance rates of HI against frequently administered oral BLs and FQs.
Methods: In a multi-centre study conducted between January and April 2007, 29 laboratories were requested each to collect 12 non-duplicate isolates from patients with CA-ENTI or CA-LRTI. Isolates were identified by standardised methods. MICs of amoxicillin (AMX), cefaclor (CEC), cefuroxime (CXM), cefixime (CFM), cefpodoxime (CPD), ciprofloxacin (CIP), levofloxacin (LVX), and moxifloxacin (MXF), erythromycin (ERY) and doxycycline (DOX) were determined

in a central laboratory by using the microdilution method according the standard ISO/FDIS 20776–1:2006. EUCAST breakpoints (BP) were applied to interpret MICs of FQ, while DIN BP were used for BLs, DOX and ERY. Production of BLA was detected by using nitrocefin discs.
Results: 290 isolates were collected. Among these, 63% were from the nasopharynges and 21% from LRT specimens. Patients ranged in age from <1 to 100 years (median 16 years). MICs50/90 obtained with the antimicrobial agents as well as the rates of susceptible (S), intermediate (I) and R isolates are shown in the Table. All isolates with reduced S to AMX (n = 24) were BLA+ (rate 8.3%). The activities of the cephalosporins were CFM = CPD > CXM > CFC. Two strains had elevated MICs of CFM and CPD (1 mg/L), though rated S. MICs of AMX for these BLA– isolates were 1 and 2 mg/L. CIP and LVX inhibited all isolates at ⩽0.06 mg/L, except one. MICs of CIP, LVX and MXF for the remaining isolate were 1, 0.5 and 1 mg/L, respectively. MXF was less active against a second isolate (MIC 0.5 mg/L), which was R to DOX.

Table. Susceptibilities of *H. influenzae* isolates to antimicrobial agents

Antimicrobial agent	MIC (mg/L)		Susceptibility (%)		
	MIC_{50}	MIC_{90}	S	I	R
Amoxicillin	0.5	2	91.7	0.3	7.9
Cefaclor	2	8	22.1	64.5	13.4
Cefuroxime	0.5	2	83.4	16.2	0.3
Cefixime	⩽0.25	⩽0.25	100	0	0
Cefpodoxime	⩽0.25	⩽0.25	100	0	0
Ciprofloxacin	⩽0.06	⩽0.06	99.7	–	0.3
Levofloxacin	⩽0.06	⩽0.06	100	–	0
Moxifloxacin	⩽0.06	⩽0.06	99.7	–	0.3
Doxycycline	⩽0.25	0.5	99.3	0.3	0.3
Erythromycin	8	⩾16	0.3	11.0	88.6

Conclusion: In Germany, BLA+ HI are still relatively uncommon and accounted for less than 10% of HI isolated from patients with CA-ENTI or CA-LRTI. Oral cephalosporins of the 2nd and 3rd generation as well as FQ retained good activity against HI.

P2055 Antibiotic resistance of *Haemophilus influenzae* isolated in invasive and non-invasive forms of children's infections in Ural region

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Objectives: Antibiotic resistance of *Haemophilus influenzae* strains is a big problem in many countries. The purpose of the current study is to determine the distribution frequency of different phenotypes antibiotic resistance of *H. influenzae* (HI) strains isolated from children with different localisation infections from 1991 to 2006 in Ural Region.
Methods: 268 non-typing HI strains obtained from clinical materials of children with the upper and lower respiratory tract inflammatory diseases, otitis media, sinusitis and 8 HI serotype "b" strains isolated from liquor and blood by osteomyelitis, meningitis and sepsis were studied for their antibiotic susceptibility. Antibiotic susceptibility was tested on Haemophilus Test Medium with the disk diffusion method (the results were checked with criteria NCCLS-CLSI) and test-systems ATB NH and ATB Haemo (ATB Expression, bioMerieux, France). Beta-lactamase was detected with chromogenic cephalosporin (cefinase-bioMerieux, France).
Results: 187 strains from 268 non-typing strains were tested to ampicillin. Only 148 (79.14%) strains from the tested 187 ones were susceptible, 15 (8.06%) were intermediate, 24 (12.8%) were resistant. Beta-lactamase was detected at 5.1% strains. It was the first time in 2003, when β-lactamase-negative ampicillin-resistant (BLNAR) 5 strains and β-lactamase-negative ampicillin-intermediate (BLNAI) 2 strains were revealed, that made 2.5% and 1% accordingly among the strains isolated from 2000 to 2005. 99 strains were tested to amoxycillin-clavulanate only 5 (5.05%) from them were resistant. 219 strains were tested to cephalosporin of the second generation only 7 (3.21%) from them were resistant. All strains were susceptible to cephalosporin of the third and fourth generation. 165 strains were tested to fluoroquinolone only 2 (1.21%) from them were resistant.
HI serotype "b" strains which had no β-lactamase were susceptible to ampicillin, amoxycillin-clavulanate, cefuroxime, cefaclor, ofloxacin, rifampicin and chloramphenicol.
Conclusion: HI serotype "b" susceptibility did not change. But resistance of non-typing HI strains changed for the investigated period: ampicillin resistance went up to 13%, resistance to amoxycillin-clavulanate and cephalosporin of the second generation appeared 5% and 3% accordingly, β-lactamase was revealed at 5.1% strains. 2.5% β-lactamase-negative ampicillin-resistant strains were revealed.

P2056 The role of antimicrobial susceptibility testing of anaerobic bacteria from clinically significant isolates

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Objectives: Anaerobic bacteria are usually saprophytic in the human body however in certain conditions they may cause severe infections. Increasing resistance to some antimicrobial agents has made susceptibility patterns less predictable.
This study aimed to determine the antimicrobial susceptibility patterns of anaerobic organisms isolated from clinical specimens.
Methods: This was a prospective study of the susceptibility data of anaerobic organisms isolated from clinical specimens from patients with suspected mixed aerobic/anaerobic infections from June 2005 until February 2007. Specimens were submitted to the microbiology laboratory where microscopy, culture and susceptibility testing to amoxycillin/clavulanate, clindamycin, metronidazole, penicllin, ertapenem, cefoxitin, ceftriaxone, chloramphenicol, and piperacillin/tazobactam were performed. The MIC (minimum inhibitory concentration) profile was determined on all isolates using the Etest® strip method and interpretation was made with reference to CLSI guidelines.
Results: Anaerobic organisms were isolated from: fluids (43), pus (41), tissues (34), blood (27), abscesses (18), bone (3), others (6), and unknown sites (8).
The most active antimicrobial agents were: chloramphenicol, piperacillin/tazobactam, ertapenem, amoxicillin/clavulanate, while metronidazole, cefoxitin, clindamycin and ceftriaxone demonstrated lesser and penicillin the least activity (see table with MIC50 and MIC90 values for all anaerobic organisms).
A total of 97 organisms were identified as belonging to the *B. fragilis* group. 100% were susceptible to amoxicillin/clavulanate and the carbapenems whereas only 86.6% were susceptible to metronidazole and 75% to clindamycin and cefoxitin.

In vitro activity of antimicrobial agents against 180 isolated anaerobic organisms

Antibiotic (breakpoint of susceptibility μg/ml)	MIC (μg/ml) 50%	90%	Range	Susceptibility (%)
Penicillin (<0.5)	12	32	0.002–256	33
Cefoxitin (<16)	3	32	0.016–256	85
Ceftriaxone (<16)	3	32	0.002–256	68
Amoxicillin/clavulanate (<4)	0.19	1	0.016–96	97
Piperacillin/tazobactam (<32)	0.19	4	0.016–256	99
Ertapenem (<4)	0.125	1.5	0.002–32	97
Clindamycin (<2)	0.19	256	0.016–256	82
Chloramphenicol (<8)	1	2	0.016–8	100
Metronidazole (<8)	0.5	16	0.016–256	89

Conclusions: This study illustrates decreasing antimicrobial susceptibility among anaerobic organisms. The elevated rates of antimicrobial resistance indicate that susceptibility testing should be performed periodically to identify emerging trends.
Piperacillin/tazobactam, ertapenem and amoxicillin/clavulanate were very active against all bacterial species, particularly to *B. fragilis* group indicating a need to modify empirical treatment for anaerobic infections.

P2057 Antibiotic susceptibility in *Helicobacter pylori* strains isolated from symptomatic Venezuelan patients

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Objective: The aim of this study was to determine the level of antimicrobial drug resistance in clinical isolate of *H. pylori* collected in Caracas, Venezuela.
Methods: Strains from 38 *H. pylori* patients with duodenal ulcers were collected from Vargas Hospital, Gastroenterology Service, Caracas, Venezuela. Primary isolation was performed on Columbia agar with 10% horse blood containing selective supplement (Isavitalex). For the E-test, a strip of each antibiotic (E-test, AB-Biodisk, Sweden) was placed upon the agar. The results were read according to the instructions of the manufacturer. All plates were incubated for 72 h at 37 ºC in microaerophilic atmosphere. Minimal inhibitory concentration (MIC) used were: Amoxicillin (>2 mg/L), Clarithromycin (>8 mg/L), Erythromycin (>2 mg/L), Tetracycline (>16 mg/L) and Metronidazole (>8 mg/L). (Etest Application Sheet).
Results: The results of MIC values of the five investigated antimicrobial agents for the 38 *H. pylori* strains were: Amoxicillin had MIC for 78% of the strains equal or less than 0.016 mg/L, MIC value of clarithromycin for 50% of the strains was <0.016 mg/L, 36% of the strains had Erythromycin MIC value of 0.016 mg/L and 55% strains had Tetracycline MIC under this value. Only 16% of *H. pylori* strains had Metronidazole MIC of <0.016 mg/L. Resistance to Metronidazole occurred in 24/38 strains (63%) while resistance of Erythromycin occurred in 2/24 strains (8%), Clarithromycin in 2/38 (5%), and Tetracycline in 3/38 (8%) of the isolated strains. None of the strains was found to be resistant to amoxicillin. In our study we observed drug multiresistance *H. pylori* isolates. Two strains were resistance to Clarithromycin + Tetracycline + Metronidazole; two strains resistant to Metronidazole + Erythromycin, and two strains with resistance to Tetracycline + Metronidazole.
Conclusions: The high frequency of resistance to metronidazole in development countries will be observed because the imidazole derivates are used frequently for the therapy of intestinal parasitic infections and gynaecological infections. In Venezuela there are not restrictions for acquisition of antibiotics and the general populations use them in an indiscriminate form. In the present study we found multiresistance strains of *H. pylori* (5.8%), where metronidazol resistance was always combined with clarithromycin or tetracycline.

P2058 15-year survey of triclosan efficacy on supragingival plaque

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Triclosan (TCN) is a broad spectrum antimicrobial formulated in dentifrices and other oral hygiene preparations for daily use. In clinical studies, TCN dentifrices demonstrate significant improvements in oral health by reducing dental plaque and gingivitis.
Objectives: This report examined the antimicrobial efficacy of TCN on human supragingival dental plaque over a 15-year period.
Methods: Adults enrolled in these studies included those who had not undergone dental procedures or antimicrobial, systemic therapies for one month prior to study participation. The study was conducted in 11 separate periods with up to 10 subjects recruited for each period. Results from 92 adult subjects were analysed for this report. Supragingival plaque was collected from the entire dentition of each volunteer and placed in pre-reduced Ringer's solution. Each dental plaque sample was sonicated and then vortexed at maximal setting for one minute to disperse bacterial clumps prior to plating dilutions on solid media containing 7.5 or 25 μg/ml TCN. Controls included dilutions of each plaque sample plated on solid media without TCN. Colonies were enumerated from all solid media following incubation.
Results: Large numbers of viable supragingival bacteria were observed from all subjects on media without TCN. By contrast, dental plaque bacteria were substantially inhibited by TCN ($p < 0.001$). Bacterial inhibition with 7.5 and 25 μg/ml TCN ranged from 96–100% and 99.6–100%, respectively. For each concentration of TCN, a one-way ANOVA examined the percent susceptible bacteria as a response between each study period that served as a variable. No differences in microbial susceptibilities were observed between each period assessed over the 15-year duration ($p > 0.3$). For each TCN concentration, regression analyses indicate no alterations or trends in efficacy ($p = 0.546$ for 7.5 μg/ml TCN and $p = 0.378$ for 25 μg/ml TCN) over time.
Conclusions: Results demonstrate the significant efficacy of TCN on supragingival plaque bacteria over the 15-year duration with no alterations in microbial susceptibility. Since study participants were not screened based on their oral hygiene habits or use of oral hygiene formulations, the results demonstrate that TCN was effective on samples from a variety of subjects. Together, the results demonstrate that TCN represents a safe and effective agent for dental plaque bacteria.

P2059 Unsuitability of MIC50, MIC90 for comparison of antibiotic potency

R. Reynolds for BSAC Working Parties on Resistance Surveillance

Objective: The potency of antibiotics is commonly compared by ranking their MIC50 or MIC90. This method is plainly unsuitable when there are distinct resistant subpopulations, but may appear attractive for unimodal MIC distributions. Its performance was investigated by simulation.
Methods: Simulations, repeated 1000 times, compared two antibiotics, A and B, each tested on all of 20, 50, 100 or 500 isolates. Simulation and analysis used the log2 scale where conventional doubling-dilution MICs (0.25, 0.5, 1, 2 mg/L, etc.) become integers (–2, –1, 0, 1, etc.). The MIC peak for A was set at 0, 0.25, 0.5 or 0.75 i.e. exactly at, or at various levels between, conventional MICs. Intrinsic variation between isolates and experimental variation were both modelled as continuous normal distributions, giving continuous 'underlying' MICs that were rounded up to integers when 'measured'. Experimental variation had an SD of 0.3, 0.4 or 0.6 (i.e. about 99%, 94% or 79% of replicate MICs within ±1 dilution). The true relative potency of A and B was constant in all isolates, differing by 0, 0.25, 0.5, 1 or 2 dilutions, but subject to experimental variation.
Results: When both drugs had equal potency, their MIC50 and MIC90 often showed apparent differences – in over 40% of simulations in some cases, even when experimental variation was low. Conversely, simple comparison of MIC50 or MIC90 could consistently fail to detect differences of 0.25 or 0.5 doubling dilutions, even with the largest sample sizes and least experimental variation, if both fell within the same doubling dilution band. The matched-pairs Wilcoxon signed-ranks test had a false-positive error rate close to an acceptable 5% level in all cases. Its power fell with increasing experimental variation (e.g. 95%, 85%, and 61% for a 0.5 dilution difference with N = 20), but increased with sample size and was unaffected by the exact position of the MICs relative to doubling dilution bands.
Conclusion: Simple comparison of MIC50 or MIC90 is unsuitable for the ranking of antibiotic potency: it performs erratically, dependent on the exact location of MICs and regardless of large sample size, and often indicates spurious differences or fails to detect real differences. The Wilcoxon signed-ranks test is safer and more powerful, and a description of paired MIC differences is more informative. More detailed understanding of the variation inherent in MIC methodology is needed for proper assessment of methods to compare MICs.

P2060 Twenty-six years review to *M. tuberculosis* resistance in Córdoba

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Objectives: Tuberculosis drug resistance is very important in control programmes. Surveillance drug resistance is therefore essential. It is reviewed the *M. tuberculosis* resistance from 1981 to 2006 in Córdoba.
Methods: A total of 1882 samples from patients with suspected tuberculose were processed. Data were collected from 1981 to 2006 in the Clinical Microbiology Service, Reina Sofia University Hospital.
Susceptibility testing to Streptomicine, Rifampicine, Ethambutol, Isoniazide, Piranizamide were determined by the radiometric method (BACTEC 460 TB) and BACTEC MGIT 960, according to the manufacturers instructions.
Results. See the table.

	Resistance percentage against *M. tuberculosis*				
	1981–85	1986–90	1991–95	1996–00	2001–05
Strains tested	202	420	499	437	324
Streptomycin	16	2	1	2	0.3
Rifampicine	3	9	9	10	7
Ethambutol	3	10	0	2	0
INH	6	20	22	7	6
Pyrazinamide	0	0	0	0	0.3

Conclusions: There is a decrease in the resistance percentage to front line antituberculose drugs during the 26 years examined.
Acording to the results exposed, resistance in Cordoba are not high, although it is remarkable that these patients are difficult to heal.

P2061 Multiple antibiotic resistance in *Campylobacter* spp. isolated from hospitalised children in Poland during 2002–2007

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Introduction: *C. jejuni* and *C. coli* have been recognised as a major cause of foodborne gastrointestinal infections in humans. Many studies have shown a significant rise in resistance to fluoroquinolones, tetracycline and erythromycin in *Campylobacter* human isolates.
Objectives: The aim of this study was to analyse the prevalence and genetic mechanisms of antimicrobial resistance in Polish *Campylobacter* strains isolated from diarrhoeal children.
Material and Methods: A total of 163 *C. jejuni* and 31 *C. coli* isolated from stool samples of paediatric patients were analysed in the study. All samples were collected from children hospitalised in 6 large paediatric hospitals in Poland from 2002 through 2007. Isolation and identification were performed according to WHO recommendations and confirmed by the PCR assay. MICs of ciprofloxacin, tetracycline, ampicillin, gentamicin, and erythromycin were determined by the E-test. The presence of the tet(O) gene was detected by PCR. Thr-86-Ile mutations in the gyrA gene were identified by MAMA PCR, and confirmed by PCR-RFLP. The PCR-RFLP method was also used for the detection of A2074C and A2075G mutations in the 23S rRNA gene responsible for macrolide-resistance.
Results: A total of 65% *C. coli* and 63% *C. jejuni* isolates were resistant to at least one antimicrobial agent tested. The highest resistance rate was observed for ciprofloxacin (57%), followed by tetracycline (20%), ampicillin (16%), and gentamicin 2%. Only one *C. jejuni* strain was resistant to erythromycin. The results of phenotypic and genetic analyses of resistance to tetracycline were fully concordant. All tetracycline-resistant isolates possessed the tet(O) gene. All *Campylobacter* isolates resistant to quinolones by the Etest had Thr-86-Ile mutations in the gyrA gene detected molecular assays. However, these mutations were also found by both molecular methods in two isolates phenotypically susceptible to ciprofloxacin and nalidixic acid. Both isolates were further confirmed by direct sequencing to contain Thr-86-Ile mutations. The highest frequency of double resistance was found for ciprofloxacin and tetracycline (22%). Triple resistance was detected in 4% of isolates tested. Multiple antibiotic resistance rose significantly between 2005 and 2007.
Conclusions: Increasing multiple-resistance among human *Campylobacter* isolates observed in Poland is an important public health concern. This imply a need for constant monitoring of resistance.

P2062 Antibiotic resistance in *Campylobacter jejuni* strains isolated from humans and poultry in the Czech Republic

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Objectives: Thermotolerant *Campylobacter* species, in particular *Campylobacter jejuni*, are among the most frequent pathogens causing alimentary zoonoses in Europe. The Czech Republic is no exception. Those were responsible for 15,700 infections reported in the country between 1 January and 31 August 2007, more than salmonella infections (12,300 cases). The work aimed at determining the resistance to selected antibiotics in both human and animal strains of *C. jejuni*.
Methods: The animal strains of *C. jejuni* were collected by cloacal swabs as part of the monitoring of thermotolerant *Campylobacter* species carried out in Czech poultry slaughterhouses. The human strains of *C. jejuni* were from rectal swabs of patients with diarrhoeal disease. The suspected isolates, both animal and human, were confirmed by the PCR. Antibiotic resistance to selected antimicrobial agents was tested by the microdilution method. The antibiotics and their cut-off values (mg/L) were as follows: erythromycin (resistant >4), ciprofloxacin (R > 1), tetracycline (R > 2), streptomycin (R > 2), gentamicin (R > 1), chloramphenicol (R > 8), ampicillin (R > 4) and oxolinic acid (R > 8). Resistance was tested in 50 strains isolated from cloacal smears in poultry and in 63 strains from human rectal swabs.
Results: The tested *C. jejuni* strains showed the highest resistance to quinolone antibiotics. Resistance to oxolinic acid was detected in 78% and 57% and to ciprofloxacin in 70% and 53% of animal and human strains, respectively. In case of ampicillin, 26% of poultry and 15% of human strains were resistant. Ten percent of animal strains but no human strains showed resistance to streptomycin. Interestingly, resistance to tetracycline was higher in human than in animal strains (11% vs. 8%, respectively). Resistance to gentamicin and chloramphenicol was 8% and 2%, respectively in poultry, but undetected in humans. In erythromycin, 6% of poultry and 2% of human strains were resistant.
Conclusion: The acquired results suggest significantly higher resistance to most antibiotics in animal (poultry) strains in comparison with human ones, the only exception being tetracycline, with higher resistance of human strains.
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P2063 Infections due to antibiotic-resistant bacteria diagnosed at hospital admission. Incidence and risk factors

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Objectives: In recent years a rising incidence of antibiotic resistant bacteria (ARB) isolated at hospital admission (HA) has been reported. Little is known about the epidemiological characteristics of patients (pts) suffering from infections due to ARB at HA. Therefore, objective of the study was to analyse risk factors for these infections.
Methods: A one-year cohort study was planned. ARB included: meticillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), extended-spectrum β-lactamase (ESBL)-producing bacteria, ciprofloxacin-resistant *Escherichia coli* or multi-drug-resistant (MDR) Gram-negative bacteria (>3 resistance to common used antibiotics) isolated within 72 hours of HA. For the risk factors

analysis, a nested case-control study was done comparing the first consecutive 100 pts with infection due to ARB to 200 randomised pts with no infections due to ARB diagnosed throughout hospital stay.
Results: In the cohort study, infections caused by ARB were diagnosed in 398 pts representing 7 cases per 1,000 hospital admissions. Twenty-two percent of isolates were ESBL-producing bacteria, 17% MRSA, 10% carbapenems-resistant *Pseudomonas aeruginosa* and 2% VRE. According to the 2007 Centers for Disease Control criteria, among 100 infections, 94% were healthcare associated and 6% were community acquired. Independent risk factors ($P < 0.05$) included: urinary catheter (odds ratio [OR] 5.7), Charlson score >3 (OR 3), antibiotic therapy within 30 days (OR 2.7) and age >60 years (OR 2.2). The presence of all risk factors was associated with the strongest evidence to rule in diagnosis of infections due to ARB at HA (likelihood ratio=12). Analysis of previous therapy revealed that glycopeptides and penicillins were predictive for meticillin resistance in *S. aureus* (area under receiver operator characteristic [ROC] curve), 65%); quinolones and penicillins for ciprofloxacin-resistance in *E. coli* (area under ROC curve, 68%); penicillins for ESBL production (area under ROC curve, 59%), for MDR-resistance in Gram-negative bacteria (area under ROC curve, 58%) and for vancomycin-resistance in enterococci (area under ROC curve, 71%).
Conclusion: Pts aged >60 yrs with clinical signs of bacterial infections at HA and urinary catheter, previous use of antibiotics, and severe underlying conditions are at increased risk for having infections caused by ARB. Recognition of these risk factors may influence the selection of empirical treatment.

P2064 Frequency of isolation of anaerobic bacteria from surgical infections and their antimicrobial resistance pattern

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Objectives: To estimate the frequency of anaerobic bacteria and their susceptibility pattern during an 18 month period(2006–2007).
Methods: 1760 specimens were studied retrospectively from intraabdominal wounds, diabetic foot ulcers, soft tissue, bone and joint infections, intrapelvic abscesses, peritoneal fluid infections, burns and other infectious sites. All samples were cultured according to conventional methods in aerobic and anaerobic conditions. Antibiotic susceptibility testing was performed by E-test(AB Biodisk, Sweden).
Results: 167(7.3%)anaerobic bacteria were isolated from 129 anaerobic cultures. 27 anaerobic cultures were polymicrobial. 42(25.1%) strains of Gram(+) anaerobic bacteria were isolated (22 *Clostridium* spp., 13 *Peptostreptococcus* spp., 4 *Eubacterium* spp., 3 *Propionibacterium acnes*) and 125(74.8%) strains of Gram(–) anaerobic bacteria (57 *Bacteroides fragilis*, 36 *Bacteroides* non-*fragilis* group, 20 *Prevotella* spp., 4 *Fusobacterium* spp., 4 *Veillonella* spp., 4 *Porphyromonas* spp.). 50 strains were isolated from soft tissue infections, 31 from intraabdominal wounds, 25 from peritoneal fluid infections, 24 from intrapelvic abscesses, 9 from diabetic foot, 9 from bone and joint infections, 8 from burns and 11 from other sites. Susceptibility testing was performed to metronidazole, penicillin, piperacillin-tazobactam, imipenem, cefoxitin, ceftriaxone, clindamycin, tetracycline and erythromycin. All isolates were susceptible to imipenem and piperacillin-tazobactam. Resistance to metronidazole was found in 4 isolates (*Prevotella* spp., *Porphyromonas* spp.). *Bacteroides* spp. isolates showed high sensitivity to β-lactamase inhibitors, metronidazole, imipenem and high resistance to clindamycin, tetracycline and erythromycin.
Conclusions: (1) Frequency of isolation of anaerobic bacteria in surgical infections was 7.3% with most common pathogen: *Bacteroides* spp. (mainly *B. fragilis*), followed by *Clostridium* and *Prevotella* spp. (2) Penicillin remains the drug of choice for clostridial infections, with metronidazole and imipenem as alternatives. High resistance to clindamycin, tetracycline and erythromycin was observed. Resistance of Gram(–) anaerobic bacteria to imipenem and metronidazole is an emerging problem.

P2065 Antibiotic susceptibility patterns of *Clostridium difficile* in a tertiary hospital in Korea from 2004 to 2005

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Objectives: Antibiotic resistance to quinolone in *C. difficile* were reported after the worldwide spread of ribotype 027 strains. Metronidazole and vancomycin MICs in *C. difficile* strains isolated in Europe (2005) were known very low. However, vancomycin and metronidazole resistance were also sporadically reported. Therefore, we investigated the rate of antibiotic resistance for the epidemiology and effective therapy of *C. difficile* in Korea.
Methods: Antibiotic susceptibility tests were performed with 91 *C. difficile* strains isolated from 2004 (50 strains) to 2005 (41 strains) in a tertiary teaching hospital in Korea. MICs of vancomycin, metronidazole, clindamycin, clarithromycin, cefotaxime, meropenem, ciprofloxacin and levofloxacin were determined using the E test (AB Biodisk, Solna, Sweden) method. Resistance was defined according to the following breakpoints: vancomycin ⩾32 mg/L, metronidazole ⩾32 mg/L, clindamycin ⩾4 mg/L, clarithromycin ⩾128 mg/L, cefotaxime ⩾32 mg/L, meropenem ⩾32 mg/L ciprofloxacin ⩾8 mg/L levofloxacin ⩾8 mg/L. PCR ribotyping was done according to the method of O'Neill. PCR assay for tcdA and tcdB genes and were performed. Van A and van B gene assay was done in strains showing increased MICs in vancomycin. Results; Of 91 *C. difficile* strains, 78 (85.7%) were toxigenic, of which 43 (56%) were toxin variant strains (tcdA-tcdB+). Most of tcdA-tcdB+ strains were same ribotype and tcdA+tcdB+ strains showed more diverse ribotypes. All the *C. difficile* strains were susceptible to vancomycin, metronidazole and meropenem. However, 11 strains (12.1%) showed increased MICs in vancomycin (over 6 mg/L) although no van A and van B genes were found. Of them 8 were tcdA-tcdB+ strains. Median MICs of vancomycin and metronidazole were 0.75 and 0.50 mg/L, respectively. Rates of resistance to clindamycin, clarithromycin, cefotaxime, ciprofloxacin and levofloxacin were 85.9%, 88.0%, 95.7%, 96.7% and 96.7%, respectively.
Conclusion: The *C. difficile* strains isolated in 2004–2005 represented high resistance to clindamycin, clarithromycin, cefotaxime, ciprofloxacin and levofloxacin regardless of the toxigenicity and ribotypes. No resistance to vancomycin and metronidazole was observed. However, a few of them showed increased MIC level in vancomycin.

P2066 Distribution of types of toxigenicity and susceptibility to antimicrobial agents among *Clostridium difficile* strains isolated in two academic hospitals in Warsaw, Poland

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Objective: This study investigates the frequence of toxins profile (A+B+CDT−, A+B+CDT+, A−B+CDT−, A−B−CDT−), and profile of susceptibility to nine antimicrobial agents among 330 *C. difficile* strains isolated from patients hospitalised in two academic hospitals (I and II between 2005–2006) in Warsaw, Poland.
Methods: Profile of toxigenicity was investigated in 330 *C. difficile* strains by phenotypic and genotypic methods. For detection of binary toxin genes PCR with specific primers pairs was used. MIC for clindamycin, erythromycin, ciprofloxacin, moxifloxacin, gatifloxacin, metronidazole, vancomycin, tetracyclin and imipenem were determined by Etest.
Results: Overall, we identified four major toxin profiles for the *C. difficile* strains under investigation. In hospital I (n = 153) were detected: 41% of A+B+CDT−, 5% of A+B+CDT+, 48% of A−B+CDT− and 6% of A−B−CDT− and among strains isolated from hospital II (n = 177) were detected: 49% of A+B+CDT−, 6% of A+B+CDT+, 40% of A−B+CDT− and 6% of A−B−CDT−. Resistance to clindamycin and erythromycin was found in 53.6% and 53.6% (hospital I) and 47.5% and 48.6% (hospital II). MLSB resistance is usually due to an ermB gene. Resistance to ciprofloxacin was found in 98% (I) and 98.3% (II), respectively. Resistance to imipenem was found in 72.4% (I)

and 74.4% (II). Resistance to tetracyclin was found in 22.6% (I and II). All A−B+CDT− belonging to PCR ribotype 017 strains were resistant to gatifloxacin and moxifloxacin. Resistance to ciprofloxacin was found in 98% (I) and 98.3% (II). All *C. difficile* strains were fully-susceptible to metronidazole and vancomycin.
Conclusion: Distribution of profile of toxigenicity and resistance to antimicrobial agents among *C. difficile* strains in two academic hospitals in Warsaw, Poland is similar. The link between toxin profiles and antibiograms is an important one given the emergence and epidemic spread of *C. difficile* strains.
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P2067 European survey of antibacterial activity against *H. influenzae* from 2006–2007: focus on fluoroquinolones

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Objectives: To document the activity of a range of antibacterial agents, including levofloxacin, against strains of *Haemophilus influenzae* from patients with community-acquired respiratory tract infections (CARTIs).
Methods: Isolates were collected between January 2006 and April 2007 in 17 European countries (156 centres) from patients with CARTIs either in the community or from patients hospitalised for <48 hours. In vitro antibacterial activity and susceptibilities of *H. influenzae* isolates were determined at a central laboratory using Clinical and Laboratory Standards Institute methodology and interpretive criteria.
Results: Of the 2669 *H. influenzae* isolates collected, 12.1% were β-lactamase positive. This varied across countries, with the highest prevalence of β-lactamase positivity found in France (24.6%) and Denmark (19.1%). Of the 2347 β-lactamase negative isolates, 4 (0.2%) were ampicillin-resistant and 36 (1.5%) showed intermediate susceptibility to ampicillin. Levofloxacin (LEV) and moxifloxacin (MOX) demonstrated the highest activity overall, with all 2669 isolates being fully susceptible to LEV and all but one isolate susceptible to MOX (Table).

Drug	All isolates (n = 2669)			ABS (n = 344)			AECB (n = 1198)		
	%S	MIC_{50}	MIC_{90}	%S	MIC_{50}	MIC_{90}	%S	MIC_{50}	MIC_{90}
AMC	99.9	0.5	1	100	0.5	1	99.8	0.5	1
CXM	98.1	1	2	98.6	1	2	97.85	1	2
CLA	71.1	8	16	74.1	8	16	70.8	8	16
SXT	80.5	0.12	8	80.8	0.12	8	78.3	0.12	8
LEV	100	⩽0.015	0.03	100	⩽0.015	0.03	100	⩽0.015	0.03
MOX	99.9	0.03	0.03	100	⩽0.015	0.03	99.9	0.03	0.03
TET	97.8	0.5	1	97.7	0.5	1	98.5	0.5	1

ABS, acute bacterial sinusitis; AECB, acute exacerbations of chronic bronchitis; AMC, amoxicillin–clavulanate; CXM, cefuroxime; CLA, clarithromycin; MOX, moxifloxacin; SXT, cotrimoxazole; LEV, levofloxacin; TET, tetracycline; MIC, minimum inhibitory concentration; S, susceptibility.

Clarithromycin was the least effective agent tested, with only 71.1% of isolates demonstrating full susceptibility. Similar results were observed when analysing isolates collected from patients with infections commonly caused by *H. influenzae* – acute bacterial sinusitis or acute exacerbations of chronic bronchitis.
Conclusions: Overall, 100% of *H. influenzae* isolates collected in this study were susceptible to LEV. Among the antibacterials tested, the fluoroquinolones LEV and MOX demonstrated the best activity, combining very low MICs and high susceptibility rates.

P2068 Previous exposure to prophylactic co-trimoxazole and resistance in bacteria from HIV-positive children: absence of resistance selection pressure of co-trimoxazole

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Introduction: Co-trimoxazole (COT) prophylaxis and ART (Antiretroviral Therapy) showed clear clinical benefit on mortality. We screened 4 years 62 HIV positive children receiving COT prophylaxis for antimicrobial resistance.
Methods: Sixty two (62) HIV/AIDS positive children from 2 orphanages in Phnom Penh (House of Family and House of Smile) on HAART (Highly Active Antiretroviral Therapy) treated with combination of stavudin, lamivudin and efavirenz for 18–42 months were examined in 3 months periods. Swabs from respiratory tract were obtained and transported in ice box within 24 hours to Reference Laboratory for Antibiotic Resistance in Slovak Republic in Nitra.
Results: Relatively high level of antimicrobial resistance – high occurrence of MRSA (Meticillin resistant *S. aureus*), PRP (Penicillin resistant pneumococci), ESBL-producing Enterobacteriaceae were observed in children before they started therapy or prophylaxis with COT and before treated with any antibiotic or antituberculotic agents for opportunistic infections. Prior exposure of COT as prevention of PCP (*Pneumocystis carinii* pneumonia) did not contribute to antimicrobial resistance in HIV children. Our results suggest that consumption of COT neither in prophylaxis nor in therapy did not contribute to increasing occurrence of Gram-positive (MRSA, PRP) or Gram-negative isolates (ESBL, CIP-R, COT-R *E. coli*, *Proteus* spp.). Similarly, concomitantly administered antibiotics (in short course, in all cases less than 10 days) in combination with COT were not significantly associated with increase of colonisation or infection with any of assessed resistotypes. Exception was amoxicillin together with COT which was associated with MRSA (P = 0.04).
Conclusion: In conclusion, neither COT alone, nor in combination with antibiotic or antituberculotic agents (RIF, INH, PZA) contribute to increased antibiotic resistance in respiratory isolates from children with HIV/AIDS. Prior exposure to oral cephalosporins and aminopenicilins insignificantly increased occurrence of PRP.

P2069 Rapid surveillance of multidrug resistance in ICU patients, by use of antibiotic-containing agar plates

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Objective: Adequate and timely administered antimicrobial treatment is the cornerstone of successful outcome in ICU infections. The aim of the study was to assess the efficacy of an in vitro method in the early screening of multidrug-resistant Gram-negative microorganisms colonising or infecting ICU patients, in view of guiding initial treatment.
Methods: In an 18-bed general ICU, surveillance cultures were performed bi-weekly for tracheal aspirates and weekly for urine and anal swabs. All specimens were directly inoculated on Mac-Conkey agar plates containing antibiotics at a concentration of 8 mg/L for Ceftazidime (CAZ), 1 mg/L for Ciprofloxacin (CIP), 4 mg/L for Imipenem (IMP) and 16/4 mg/L for Piperacilline/Tazobactam (PT). The method was evaluated for a six week period and was compared to standard microbiological procedures (disk diffusion method, CLSI 2007 breakpoints of resistance). For COL BSAC breakpoint for disk diffusion method was used. Tracheal aspirates were processed quantitatively. Discordant results were categorised as major error when a resistant isolate did not grow on the antibiotic containing agar, appearing to be sensitive, and as minor when the converse occurred.
Results: A total of 604 Gram-negative isolates were compared; 208 *Acinetobacter baumannii*, 138 *Pseudomonas aeruginosa*, 27 *Stenotrophomonas maltophilia* and 271 enterobacteriaceae (115 *Klebsiella pneumoniae*). Concordance of the two methods, minor and major errors of the tested antibiotic-containing agar plates are displayed in table 1. Specimens with high dense inocula did not affect the sensitivity of the

method for all antibiotics except PT (p 0.006). The presence of multiple microorganisms in the specimen reduced significantly the sensitivity of CAZ- and PT-enriched plates (p 0.005 and 0.014 respectively). COL-enriched plates displayed unacceptably reduced concordance with standard method, probably reflecting the poor diffusion of the antibiotic in the agar.

In 12 enterobacteriaceae the final result of CAZ disk was reported discordant to that measured by diameter due to an ESBL phenotype, whereas this was also the case for IMP disk among 30 isolates of *Pseudomonas aeruginosa* and enterobacteriaceae with MBL phenotype.

Table 1

	Antibiotic containing agar plate				
	CAZ	CIP	IMP	PT	COL
Concordance (%)	75.9	72.3	72.4	69.8	33.3
Major errors (%)	10.09	14.74	14.4	16.6	6.29
Minor errors (%)	13.07	12.75	12.6	12.08	57.8

Conclusions: The use of antibiotic-containing agar plates demonstrates satisfactory concordance with standard microbiological procedures and could prove useful in the early guidance of initial treatment of ICU patients with infections.

P2070 A real-time PCR and pyrosequencing strategy for screening of penA mosaic alleles and diagnosis of reduced susceptibility to expanded-spectrum cephalosporins in *Neisseria gonorrhoeae*

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Objectives: High levels of resistance in *N. gonorrhoeae* (GC) to all traditional gonorrhoea antibiotics are today prevalent globally. Furthermore, emergence of reduced susceptibility and resistance to azithromycin and, recently, to expanded-spectrum cephalosporins forms a serious concern worldwide.

Reduced susceptibility to expanded-spectrum cephalosporins such as ceftriaxone and cefixime (cefI) is associated with penA gene polymorphisms and, especially, presence of penA mosaic alleles, which encode an altered penicillin-binding protein 2 with decreased affinity to cephalosporins.

The aims of the present study were to develop a rapid, objective and precise screening method for detection of penA mosaic alleles and, accordingly, diagnosis of reduced susceptibility to expanded-spectrum cephalosporins such as ceftriaxone and cefixime in GC.

Methods: An "in silico" examination of all available GC penA sequences identified two polymorphic nucleotide sites that are altered in all penA mosaic alleles of CefI isolates, i.e. C1512G and C1529T, but not in any cephalosporin susceptible wild type penA isolates.

For development of a penA mosaic alleles screening method, 18 GC CefI isolates (ceftriaxone MIC$\geqslant$0.094 mg/l and cefixime MIC$\geqslant$0.19 mg/l) and two penA wild type isolates were used. These isolates were earlier comprehensively characterised, genetically and phenotypically (Lindberg et al. AAC 2007).

For identification of GC CefI isolates, our previously published *N. meningitidis* PenI screening method based on real-time PCR and pyrosequencing (Thulin et al. AAC. In press) was used with minor modifications.

Results: Pyrosequencing allowed rapid determination of 40–50 nucleotides in up to 96 different sequences and were in concordance with conventional Sanger sequencing. The two CefI specific sites were effectively identified and evidently comprised reliable markers for penA mosaic alleles and, accordingly, reduced susceptibility to ceftriaxone and cefixime.

Conclusions: Real-time PCR and subsequent pyrosequencing for screening of penA mosaic alleles in GC is an effective method for genetic detection of reduced susceptibility to expanded-spectrum cephalosporins such as ceftriaxone and cefixime. This method enables rapid, objective, sensitive, specific and high throughput screening, most probably also directly of clinical samples.

P2071 Study of *Neisseria gonorrhoeae* during a seven year period in a Spanish teaching hospital

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Introduction: The aim of this study was to analyse the antimicrobial susceptibility of *Neisseria gonorrhoeae* isolated from men with urethritis and to study the incidence of the microorganism during a seven year period in a Spanish teaching hospital.

Methods: One hundred and ninety three strains of *Neisseria gonorrhoeae* were isolated from 683 samples of urethral exudates, received at the Microbiology Department (Hospital Universitario de la Princesa, Madrid) from January 2001 to November 2007.

Urethral exudates were collected by standard procedures. They were examined by Gram stain and inoculated on Modified Thayer-Martin medium and Chocolate agar, and incubated at 37º in 10% CO2 until 48 hours. Penicillin, ciprofloxacin, tetracycline, ceftriaxone and spectinomycin suceptibility tests were performed using disc diffusion method.

Results: The percentage of positive cultures according to the number of samples received were 19.41%, 20.68%, 23.45%, 25.64%, 32.25% and 39.83% in 2001, 2002, 2003, 2004, 2005 and 2006 years respectivily. The susceptibility rate was 74.28% for penicillin, 99.42% for ceftriaxone, 72.14% for tetracycline, 63.12% for ciprofloxacin and 98.48% for spectinomycin. The susceptibility to ciprofloxacin decreased from 93.33% in the period of 2001–2003 to 67.93% in 2005–2007 years. Only one and two strains were resistant to ceftriaxone and spectinomycin, respectivily.

Conclusions: According to our work, resistance to penicillin, tetracycline and ciprofloxacin is high and can not be recommended for the treatment of gonorrhoea in our area. Ceftriaxone and spectinomycin should be considered the antimicrobial of choice. Quinolone resistance increased considerabily during the period of this study. Regarding to the rate of positive cultures, the number of gonorrhoea cases have increased from 19.41% cases in 2001 to 39.83 cases in 2006, probably due to changes in surveillance practices.

P2072 Susceptibility patterns of *Ureaplasma urealyticum* isolates, obtained form urogenital samples

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Introduction: *Ureaplasma urealyticum* can be isolated from the human urogenital tract as common comensal or pathogen. It can cause various pathologies such as non-gonococcal urethritis, bacterial vaginosis, cervicitis, endometritis or pelvic inflammatory disease. The aim of this study was to analyse antimicrobial susceptibility of *Ureaplasma urealyticum* isolated from urogenital samples.

Methods: All the urogenital samples received at the Department of Microbiology (Hospital Universitario de la Princesa, Madrid) from January 2005 to November 2007 were collected into the selective broth and transported to the laboratory. The diagnostic method was using Mycoplasma IST 2 kit (BioMerieux), a biochemical comercial method. Susceptibility to doxycycline, josamycin, azithromycin, erythromycin, clarithromycin, tetracycline, ofloxacin and ciprofloxacin were tested using Mycoplasma IST 2 kit.

Results: Six hundred and fifty nine urogenital samples were positive to *Ureaplasma urealyticum* in this period. 42.0% were obtained from women samples (165 endocervical exudates and 112 urine samples) and 58.0% were obtained from men samples (183 protatitis protocols, 92 uretral exudate and 107 urethritis protocols). The susceptibility rate was 89.0% for doxycycline, 85.7% for tetracycline, 11.9% for ofloxacin,

2.1% for ciprofloxacin, 38.7% for erythromycin, 38.4% for azitromycin, 72.9% for clarithromycin and 73.7% for josamycin. In the previous years, from January 2001 to December 2004, the susceptibility rate to ofloxacin and erythromycin were 70.7% and 62.5% respectivily.
Conclusion: According our review, the resistance to quinolones was higher than resistance to other antibiotics tested. Ofloxacin resistant strains have increased from 29.3% in the years 2001–2004 to 88.1% in the years 2005–2007. The resistance rate to erithromycin also was higher in the years 2005–2007 than in the previous years.

P2073 Reversibility of antimicrobial resistance in respiratory isolates in HIV-positive Cambodian children after 42 months of HAART

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Objectives: Aim of this study was to assess, if restauration of the immune system after 42 months treatment with HAART in Cambodian children has an impact on antibiotic resistance and its reversibility.
Methods: Study participants were HIV positive Cambodian children treated with HAART stavudine, lamivudine and nevirapine or efavirenz. Respiratory tract isolates (nose, pharyngeal, ear svabs) from cambodian previously ART naive children 3–11 years old were assessed every 3 months within 42 months of HAART.
Results: Analysing relationship between duration of HAART, and colonisation with any specific resistance pattern, MRSA appeared to emerge after 6–12 months of HAART in comparison to pre-HAART period (was 90–93% after 9–12 months vs 50% in HAART naive) and slightly decreased to 63% after 42 months. Proportion was biphasic. Presence of multiresistant *Klebsiella*, and *Enterobacter* spp. was high already at baseline and in first months of HAART and the proportion of multiresistant Gram-negative bacteria (MR GNB) decreased later to 14.4% after 42 months. Susceptibility of both Gram-negative and Gram-positive bacteria showed biphasic but increasing tendency. Proportion of MR GNB decreased from 21/23 (90%) in the first 6 months of HAART, to 0–11% in those receiving HAART for 15–18 months and to 14% after 42 months of HAART. Reversibility of MR in GNB took 15–18 months. However, the baseline of resistance in GNB were relatively high. Proportion of MRSA increased from 50–55% in first 6 months to 93–85.7% after 9–18 months but than decreased. Emergence of MRSA was slower. Reversibility of MR in *St. aureus* was longer and took approximately 24–30 months. Ratio of Gram-positive to Gram-negative decreased from 1:3.9 (HAART naive) to 1:1.2 (42 month of HAART).
Conclusion: Reversibility of resistance among isolates from respiratory system was probably due to the reconstitution of their immune system due to the HAART and therefore less exposition with therapeutic ATB. In MRSA, the reversibility of resistance took 15–21 months and was slower than in MR Gram-negative bacilli (*Klebsiella*, *Enterobacter*) where the increase of susceptibility (and the decrease of resistance) took 9–12 months. Prophylactic administration of cotrimoxazol 3× weekly did not affect the reversibility of resistance and seems to be less promotive for antibiotic resistance.

P2074 Susceptibility patterns of *Helicobacter pylori* paediatric strains in Spain

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Objective: *Helicobacter pylori* is a Gram-negative microaerophilic bacillus found in the human stomach and is associated with a wide range of digestive diseases. *H. pylori* resistance is an important factor in the efficacy of the treatment. The aim of this study was to determine the prevalence of antimicrobial resistance in *H. pylori* strains from paediatric patients. Isolates were tested against metronidazole, clarithromycin, ciprofloxacin, amoxicillin and tetracycline.
Methods: 193 samples were collected from gastric biopsies obtained by endoscopy to symptomatic children from January 2006 to November 2007. 83 out of 193 were positive for *H. pylori*. Biopsies were cultured according to standard microbiological procedures. The susceptibility was determined by E-test using 5% sheep blood agar (3–5 days incubation at 37º C in 10% CO2). Minimum inhibitory concentration was determined as the lowest concentration of drug able to inhibit visible growth, based on the CLSI and other previously published data.
Results: The resistance rate was 40.2% for metronidazole, 48.4% for clarithromycin and 11.4% for ciprofloxacin. All isolates of *H. pylori* were susceptibility to amoxicillin and tetracycline.

Table 1. Resistance rate

Antibiotic	Total	Resistant strains	%
Metronidazole	97	39	40.2
Clarithromycin	97	47	48.4
Ciprofloxacin	96	11	11.4
Amoxicillin	97	0	0
Tetracycline	97	0	0

Conclusions: We found a high rate of clarithromycin and metronidazole resistance in our study. Due to the fact that clarithromycin resistance is specially critical and induce treatment failure, the use of this agent in empirical therapy should not be adviced in the treatment of *Helicobacter pylori* in our paediatric population. The data limit the therapeutic arsenal in *Helicobacter pylori* infection in children.

P2075 In vitro activity of tigecycline and other antimicrobial drugs against selected pathogens isolated in Mexico

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Objective: A multi-national, multi-centre susceptibility survey (TEST) was established to assess the in vitro efficacy of tigecycline and other antimicrobials against relevant aerobic pathogens. Here we report the results of 1,280 isolates from 6 hospitals at the three main Mexican cities
Methods: The survey included fresh isolates of selected pathogens, identified as causative of infection; only one isolate per patient was included, and no more than 25% of the sample came from urinary tract infections. Minimal inhibitory concentrations (MIC) were assessed by microdilution to ampicillin (AM), amoxycillin-clavulanate (AC) piperacillin-tazobactam (PT), levofloxacin (LV), ceftriaxone (CX), minocycline (MC), imipenem (IM) and tigecycline (TG) for all isolates (except TG for *Acinetobacter* and *Pseudomonas*), and linezolid (LZ), vancomycin (VA) and penicillin (PN) for Gram-positives, or cefepime (CF), ceftazidime (CZ) and amikacin (AK) for Gram-negatives. Data were recorded in forms and compiled in a global database; strains were stored and shipped to a central lab for further testing (ESBL production by enteric bacteria, using ceftazidime/cefotaxime+clavulanate disks; and meticillin resistance in *S. aureus*, using cefoxitin disks).
Results: Most relevant resistance prevalences include: 48% of *S. aureus* are MRSA; only one enterococcus strain was LZ-R; 12.5% of *S. pneumoniae* were PN-R; 36% of *E. coli* and 28% of *K. pneumoniae* were ESBL-producers; resistance to carbapenems was common in non-fermentative Gram-negatives, but absent in enteric bacteria. Only one strain (*K. pneumoniae*) out of 1,001 tested were resistant to TG; even MC-R strains, were susceptible to TG. Resistance to LV was linked to MRSA (94%, vs. 4% among MSSA), and to ESBL-producers: 65% of *K. pneumoniae* (vs. 31% total) and 99% of *E. coli* (vs. 70% total).
Conclusions: Dangerous multi-resistant organisms (MRSA, VRE, ESBL-producers) were found at high rates among Mexican pathogens, some linked to unrelated resistance phenotypes, which could foster resistance by co-selection. Non-fermentative bacilli were often resistant to drugs of choice (AK, cephalosporins, carbapenems). Linezolid was, in vitro, the most effective drug against Gram-positives, and tigecycline the most effective drug overall, justifying its position against intra-abdominal

and skin infections. These antibiotics should be used carefully to preserve their efficacy.

Species	% Resistant											
	AM	AC	PT	CX	CZ	LE	LZ	VA	IM	AK	TG	LV
S. agalactiae	0			0		0	0	0			0	
E. faecalis	0			–		30	0	0			0	
E. faecium	85			–		85	0	36			0	
S. aureus	83			41		48	0	0			0	
E. coli	91	28		50	20	70				3	0	
E. cloacae	95	97		25	42	15				10	0	
K. pneumoniae	91	24		–	30	32				7	1	
P. aeruginosa			29		39				32	20		39
A. baumannii			51		72				19	47		51

P2076 US survey on resistance among the *Bacteroides* species for 2005–2007

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Objectives: A US based survey to evaluate the in vitro susceptibility of the *Bacteroides* species to commonly used and newer antibiotics, to determine association of resistance with species, and to evaluate trends over time was continued.

Methods: The MICs of over 1,000 isolates of *Bacteroides* species were determined by agar dilution method following CLSI (NCCLS 2004) recommendations. Incubation was for 48 h in an anaerobic chamber.

Results: Imipenem, meropenem, ertapenem and piperacillin/tazobactam continue to demonstrate the highest in vitro activity against these pathogens. Doripenem, first tested in the survey in 2006 had 100% in-vitro activity against all isolates except for 1 isolate of *B. fragilis*. The resistance range to the carbapenems and piperacillin/tazobactam in *B. fragilis* was from 0.9–2.3%. Resistance to ampicillin:sulbactam had yearly increases and was 21.1% for B. distasonis in 2006. Resistance for tigecycline was 2.8% for *B. fragilis* in 2006 but resistance was 9.1% in B. uniformis, 9.7% in B. thetaiotaomicron and 5.3% in B. ovatus.

The highest resistance rates among the antibiotics tested were seen with clindamycin, moxifloxacin and trovafloxacin; overall resistance to most species was about 30% to these compounds, and exceeded 50% in isolates of B. vulgatus. Interestingly, Linezolid was more active than clindamycin. Three isolates of metronidazole resistant *B. fragilis* from one patient were seen in 2007.

Conclusions: Our results indicate that resistance to antibiotics is greater among the non-*fragilis* species and are increasing markedly among species with low frequency of isolation, such as B. caccae and B. uniformis. Doripenem showed activity similar to imipenem or meropenem. Tigecycline remained quite active. Resistance to the fluoroquinolones, moxifloxacin and trovafloxacin, continues to increase among most of the species of the group. The emergence of resistance among the non-*fragilis* species demonstrates the need for speciation of these organisms, and clinician awareness to species/drug resistant associations.

P2077 Decreasing β-lactamase production and trimethoprim-sulfamethoxazole resistance in *Haemophilus influenzae* in Spain (1997–2007)

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Objectives: 1) to know the current antimicrobial susceptibility of *Haemophilus influenzae* isolates in 2007 in comparison with a control group of similar strains isolated in 1997, and 2) to analyse trends on antimicrobial susceptibility in relation to the evolution of antibiotic consumption in the community.

Methods: A total of 197 clinical isolates of *H. influenzae* were studied; 109 were isolated in 2007 and 88 in 1997 (control group). In both periods, they were consecutively isolated from January to March from the same Spanish geographic area (Hospital Gregorio Marañón, Madrid), which has not suffered relevant changes over time as regards to population coverage. The antibiotic susceptibility was determined by the broth microdilution method according to the CLSI guidelines. Antibiotic consumption was determined according to the ATC/DDD methodology.

Results: No statistical significant differences were found between 2007 and 1997 isolates in relation to their clinical source, patient's age and microbiological data. Most of them were from children (56% in 2007 and 55.7% in 1997) and respiratory infections (93.6% in 2007 and 86.4% in 1997). A total of 34 (31.2%) of the isolates were resistant to amoxicillin in 2007 and 33 (37.5%) in 1997; the percentage of β-lactamase production were 17.3% in 2007 and 33% in 1997 ($P = 0.01$, OR 0.43, 95% CI 0.22–0.83). Resistance to amoxicillin in β-lactamase negative isolates were 13.8% in 2007 and 5.7% in 1997. The proportion of isolates with an azithromycin MIC $\geqslant 2$ mg/l was 87.2% in 2007 and 66% in 1997 ($P = 0.0005$, OR 3.50, 95% CI 1.72–7.20). The prevalence of cotrimoxazole resistance decreased, from 50.1% (1997) to 34.8% (2007) ($P = 0.04$, OR 0.53, 95% CI 0.30–0.95). The antibiotic consumption at the community level of amoxicillin + β-lactamase inhibitor and azithromycin increased up to 50%.

Conclusions: Resistance to amoxicillin due to β-lactamase production decreased 15.7% in 10 years in Spain. However, the prevalence of β-lactamase negative amoxicillin-resistant isolates increased by a percentage of 8.1%. A good correlation was found between antibiotic consumption trends in the community and variation on antimicrobial susceptibility.

P2078 Beta-lactamase activity against *H. influenzae* exhibiting amoxicillin (with or without clavulanate) resistance phenotypes/genotypes

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Objective: To explore the in vitro activity of amoxicillin/clavulanate (AMC), cefuroxime (CXM), cefdinir (CDR), cefotaxime (CTX), and cefditoren (CDN) against 150 isolates belonging to the β-lactamase negative ampicillin (AMP)-resistant (BLNAR) and β-lactamase positive AMC-resistant (BLPACR) *H. influenzae* resistance phenotypes.

Methods: In vitro susceptibility of 126 BLNAR (64 with ampicillin MIC=2 mg/l, and 62 with MIC$\geqslant$4 mg/l) and 24 BLPACR (19 with AMC MIC=4 mg/l, and 5 with AMC $\geqslant$8 mg/l) isolates from 6 Spanish hospitals in 2004–2007, was determined by microdilution following CLSI recommendations. CLSI susceptibility breakpoints (mg/l) considered were: AMP $\leqslant$1; AMC $\leqslant$4/2, CXM $\leqslant$4, CDR$\leqslant$1, and CTX $\leqslant$2. No CLSI breakpoints are defined for CDN. Mutations in the ftsI gene encoding PBP3 were determined by PCR amplification and direct sequencing.

Results: MIC50 (mg/l), MIC90 (mg/l), and % susceptibility (%S) are shown in the Table.

A total of 90.5% BLNAR (AMP MIC $\geqslant$2 mg/l) and 79.2% BLPACR (AMC MIC $\geqslant$4 mg/l) phenotype isolates tested exhibited single or multiple mutations in the ftsI gene, being N526K (present in 112

isolates), V545I (103 isolates), D350N (83 isolates) and A502T/V (69 isolates) the most frequent.
Conclusion: The range of intrinsic activity in terms of MIC90 values was: CDN > CTX > AMC = CDR > CXM. CDN exhibited potent activity (2–4 times higher than CTX) against all *H. influenzae* isolates exhibiting a BLNAR/BLPACR phenotype that showed low susceptibility rates to AMC, CDR and CXM.

	BLNAR (n = 126)				BLPACR (n = 24)	
	AMP MIC = 2 (n = 64)		AMP MIC ⩾ 4 (n = 62)		AMC ⩾ 4	
	MIC_{50}/MIC_{90}	%S	MIC_{50}/MIC_{90}	%S	MIC_{50}/MIC_{90}	%S
AMC	2/4	65.6	2/4	66.6	4/8	0.0
CXM	4/16	56.2	4/16	51.6	4/8	50.0
CDR	2/4	48.4	2/8	39.3	1/4	50.0
CTX	0.06/0.25	98.4	0.06/0.5	98.4	0.06/0.12	100
CDN	0.06/0.06	–	0.06/0.12	–	0.03/0.06	–

Epidemiology of antimicrobial resistance among Gram-negatives

P2079 Resistance to carbapenems and phenotypic detection of metallo-β-lactamases in *P. aeruginosa* clinical isolates

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The most common cause of bacterial resistance to β-lactam antibiotics is the production of β-lactamase. Among these, metallo-β lactamases (MBLs) are responsible for carbapenem resistant *P. aeruginosa* strains. Emerging resistance to carbapenems limits therapeutic options, thus rapid detection of MBLs is crucial for patient treatment. The aim of this study was to determine the prevalence of carbapenem-resistant *P. aeruginosa* strains producing MBL, by using screening tests.
Methods: A total of 115 non-duplicated clinical isolates of *P. aeruginosa* collected in our hospital from January 2005 to June 2007, were investigated for susceptibility to carbapenems. The identification and the determination of antibiotic sensitivity was performed by using the Wider system (Soria). Carbapenem resistance was confirmed by E-test (AB Biodisk). All the carbapenem resistant isolates were tested by MBL E-test and the imipenem–EDTA double-disk-synergy test (DDST), for a possible metallo-β-lactamase production.
Results: Nineteen strains were found to be resistant to carbapenems. Fifteen strains were resistant to both carbapenems, three strains had MIC < 4 for imipenem and MIC 4–8 for meropenem and one strain had MIC 4 to imipenem but was sensitive to meropenem. Resistance to other antibiotics was: ciprofloxacin 60%, ceftazidime 53%, gentamycin 73%, tobramycin 46%, piperacillin 53% and piperacillin/tazobactam 33%. Ten out of the nineteen carbapenem resistant strains were producers of MBLs. Four strains that were resistant only to one carbapenem, were negative for MBL production, using both tests. From the remaining fifteen resistant to both carbapenems strains, five were negative for MBL, and seven strains were found to be producers of metallo-β-lactamases by using both, E-test MBL and the imipenem-EDTA-DDST. Three strains were undetermined for MBL production using E-test but were positive using the EDTA-DDST. The most active antibiotics against MBL strains were: colistin 100% sensitive strains, piperacillin/tazobactam 80% and aztreonam 60%. The least active were: gentamicin (80% resistant strains) and ceftazidime (50%).
Conclusion: The most active β-lactam antibiotic against MBL *P. aeruginosa* strains was piperacillin/tazobactam. The study showed that MBL screening tests are simple and easy to perform as routine diagnostic tests for the detection of MBL-production. DDST is more sensitive and less expensive than E-test MBL.

P2080 Detection of metallo-β-lactamases in Enterobacteriaceae clinical isolates

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Metallo-β-lactamases (MBL) have been the focus of increasing research during the last years due to their ability to hydrolyse most β-lactams, including carbapenems. Acquired MBLs have emerged among nosocomial *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates and more recently among several enterobacteriaceae isolates. In the present study we have investigated the emergence of MBL-producing enterobacteriaceae strains from clinical specimens.
Methods: total of 314 non-replicate enerobacteriaceae isolates (111 isolates of *Klebsiella pneumoniae*, 101 *Proteus mirabilis* and 99 *Enterobacter cloacae*) were obtained from specimens of patients with nosocomial infections, between June 2005 and July 2007. identification and susceptibility testing was performed with the Wider system (Soria). The carbapenem resistance was confirmed by E-test (AB Biodisk). All isolates with reduced susceptibility to carbapenems were phenotypically tested for MBL production by using the imipenem-EDTA synergy test (DDST) and the E-test MBL assay (AB Biodisk). The detection of various bla gene types on these isolates was performed by PCR amplification.
Results: In total, seventeen carbapenem-resistant isolates, three isolates of *K. pneumoniae*, seven of *E. cloacae* and seven of *P. mirabilis*, were recovered. Eleven strains had $MIC^3$8 to both carbapenems, four strains had MIC 8 for imipenem and MIC < 4 for meropenem and two strains had MIC 16 for meropenem and were sensitive to imipenem. The imipenem-EDTA DDST and the MBL E-test showed a positive result only for three strains, including one strain for each enterobacteriaceae species. The *E. cloacae* and *P. mirabilis* MBL-positive strains were isolated from urine samples of two patients from medical wards and the *K. pneumoniae* positive strain was isolated from blood of an intensive care unit patient. The strains were resistant to all β-lactams and only one of them showed an intermediate sensitivity to aztreonam. With these strains a PCR was carried out using primers for VIM- and IMP-type genes which confirmed the presence of bla VIM genes.
Conclusion: The prevalence of MBL in enterobacteriaceae strains is low. In our hospital, these strains are the first enterobacteriaceae species producing bla VIM genes. Continuous surveillance and detection of MBL production are needed when the enterobacteriaceae strains show a suspected profile of resistance.

P2081 Study of metallo-β-lactamase-producing *S. marcescens* isolates from a children's intensive care unit

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Objectives: The aim of this study was to determine the occurrence of MBLs in five isolates of *S. marcescens* resistant to carbapenems that had been isolated from the NICU of the children's hospital in 2007.
Material and Methods: We study 5 strains (samples: 2 BAS, 2 conjuntival and 1 umbilical) of *S. marcescens* isolates from 4 newborns receiving meropenem.
The identification and sensitivity analyses were done using Wider panels MIC/ID Gram-negatives (Soria Melguizo, S.A.®).
Sensitivity to aztreonam, imipenem and meropenem was determined by E-test.
The MLBs phenotype was confirmed with EDTA-imipenem/imipenem E-test and diffusion in Mueller-Hinton agar with an imipenem and EDTA containing disks.
Final molecular characterisation was made by ERIC-PCR, RAPD and PCR using specific primers for different metallo-β-lactamases families.
Results: All the isolates were resistant to all β-lactams, except Aztreonam and showed resistance to trimethoprim-sulfametoxazole.
The MIC to imipenem was >8 μg/mL.
The E-test of imipenem/imipenem-EDTA showed an inhibition of the metaloenzime over more than 4 dilutions

Analyses by PCR showed the presence of a β-lactamase of the VIM family of metalloenzymes in a class I integron. Fingerprinting by PCR showed that the MBL producers belonged to different strains.
Conclusions: This is the first description in Spain of *S. marcescens* producer of metallo-β-lactamase in a clinical isolates
The fact that different strains carried the same MBL gene indicates that the integron has repeteadly entered in *S. marcescens* and has probably been selected by the use of meropenem. Appropriate control measures, including introduction of MBL screening in *S. marcescens* MIC > 1 μg/ml to meropenem, are necessary in order to prevent wider dissemination of these strains.

P2082 VIM-1 and CTX-M-9 in *E. cloacae*, *K. pneumoniae* and *K. oxytoca* clinical strains

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Objectives: The carbapenem-hydrolysis β-lactamases (CHBL) begins to be clinically relevant. This resistance poses two major challenges, detection in the microbiology laboratory and the choice of a valid antibiotic therapy.
We describe 12 Enterobacteriaceae isolates with carbapenemase activity isolated at Hospital del Mar (Barcelona, Spain) from December 2006 to May 2007. Two of them also produced the CTX-M-9 ESBL.
Methods: Identification and susceptibility of isolates were done by MicroScan system (Urine ComboS1 and NC36 panels). To confirm carbapenem resistance, imipenem and ertapenem disc diffusion, imipenem and imipenem+EDTA E-test and the cloverleaf test were performed. PFGE using XbaI restriction enzyme was done to disregard clonality between the different isolates. The β-lactamases genes were characterised by PCR and sequencing. The incompatibility group of the CHBL carrying plasmid was determined by incompatibility group determination, and was made by rep-typing PCR, S1 digestion, PFGE, Southern Blot and hybridisation with specific probes for 18 different incompatibility groups.
Results: Seven *Enterobacter cloacae*, 2 *Klebsiella pneumoniae* and 3 *Klebsiella oxytoca* isolates showed a VIM-1 metallo-β-lactamase. Four clones were found among the *E. cloacae* isolates: PFGE-I with 3 strains, PFGE-II with two strains and PFGE-III and IV with one isolate each. The three *K. oxytoca* strains were clonal. The 3 *E. cloacae* with PFGE pattern I and three *K. oxytoca* strains also presented the CTX-M-9 enzyme. Two plasmids of 38 bp and 75 bp hybridised with the carbapenem resistance probe, but we were unable to define the incompatibility group. The blaCTX-M-9 was found in a HI2 plasmid of 290 pb.
All isolates were classified as susceptible by MicroScan system; only two *E. cloacae* had slightly higher MICs values (IMP 4 μg/ml and ERT 2 μg/ml). The disc diffusion method correctly classified *E. cloacae* isolates as intermediate or resistant but were not the case for *Klebsiella* isolates. A slight synergy effect was observed with EDTA in the E-test. The cloverleaf test was positive in all isolates, but not proved useful in *E. cloacae* due to the interference with his chromosomal betalactamase.
Conclusions: It is important for laboratories to be aware of carbapenem resistance detection because is elusive in some cases. Additional tests like disk diffusion, E-test, cloverleaves are mandatory when treatment relies on these antibiotics.

P2083 Frequency and antibiotic susceptibility of extended-spectrum β-lactamase producing Enterobacteriaceae isolated from urinary tract infections in a two-year period

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Objectives: To study the isolation frequency and antibiotic susceptibility of ESBL-producing enterobacteriaceae strains isolated from hospitalised and community patients with urinary tract infections (UTI).
Methods: During a two-year period (2006–2007), 976 strains of *Escherichia coli* and 142 strains of *Klebsiella pneumoniae* were isolated from midstream urine samples in our microbiology laboratory. Identification to the species level was performed with the VITEK TWO system (Bio-Merieux) and susceptibility to antimicrobial agents was tested by the disk diffusion method according to CLSI recommendations. Detection of ESBL expression was performed by two methods, the double-disk synergy (DDS) test and the VITEK TWO card MIC/AST for ESBL. From the strains tested, 696 (584 *E. coli* and 112 *K. pneumoniae*) were isolated from hospitalised patients and 422 (392 *E. coli* and 30 *K. pneumoniae*) from community patients with UTI.
Results: ESBL production was determined in 51 out of 1118 strains of Enterobacteriaceae (4.6%). The ESBL phenotype was detected in 3.4% (33 out of 976) strains of *E. coli* and 12.7% (18 out of 142 strains) of *K. pneumoniae* urine isolates. For *E. coli*, ESBL production was detected in 30 out of 584 strains isolated from hospitalised patients (5.1%) and 3 out of 392 patients of the community (0.8%). For *K. pneumoniae*, ESBL production was detected in 15 out of 112 strains from hospitalised patients (13.4%) and 3 out of 30 strains from community patients (10.0%). The antibiotic susceptibility rates for the ESBL-producing strains were for *E. coli* and *K. pneumoniae* respectively: imipenem 100%-100%, gentamicin 57.6%-66.7%, amikacin 78.8%-38.9%, netilmicin 69.7%-44.4%, tobramycin 48.5%-11.1%, chloramphenicol 57.6%-44.4%, ciprofloxacin 24.2%-11.1%, norfloxacin 24.2%-11.1%, tetracycline 18.2%-50.0%, trimethoprim/sulfamethoxazole 24.2%-5.6%. ESBL production and fluoroquinolone resistance was detected in 25 out of 33 (75.8%) strains of *E. coli* and 16 out of 18 (88.9%) strains of *K. pneumoniae*.
Conclusions: ESBL-producing bacteria are becoming an increasing therapeutic problem not only in hospitalised but also in community patients. High resistance rates to quinolones were observed among these strains, but carbapenems had a good activity. Monitoring of ESBL production and antibiotic susceptibility testing are necessary to avoid treatment failure in patients with urinary tract infections.

P2084 Bloodstream infections due to extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae*: risk factors and clinical outcome

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Objectives: The bloodstream infections (BSIs) caused by extended-spectrum β-lactamase (ESBL) producing *Escherichia coli* and *Klebsiella pneumoniae* are a worlwide problem for hospitalised patients, since they markedly increase the rates of treatment failure and mortality. The purpose of this study is to evaluate the risk factors and clinical outcomes of BSIs by ESBL-producing *E. coli* and *K. pneumoniae*.
Methods: We analysed the episodes of *E. coli* and *K. pneumoniae* bacteraemia in Baskent University Adana Research and Practice Center between January 2006 and August 2007. The antibiotic susceptibilities and ESBL production of each isolate was determined by the disk diffusion method, employing the criteria of Clinical and Laboratory Standards Instutute. Descriptive data were extracted from the medical records of the patients with *E. coli* and *K. pneumoniae* bacteraemia. The data collected included age, sex, underlying disease, comorbit conditions, primary site of infection, severity of illness as calculated by the Acute Physiology and Chronic health Evaluation (APACHE) II score, duration of hospital stay, antimicrobial regimen, and any antimicrobial therapy in the 30 days prior to onset of bacteraemia. Each BSI episode was classified based upon Centers for Disease Control and Prevention guidelines. The major study outcome was survival status, which was calculated as the mortality rate within 30 days after the onset of bacteraemia.
Results: A total of 118 episodes of *E. coli* bacteraemia and 41 episodes of *K. pneumoniae* bacteraemia were identified. Of these, 40.7% (48/118) of the *E. coli* strains and 31.7% (13/41) of the *K. pneumoniae* strains were ESBL-producing organisms. Results are summarised in tables 1 and 2.
Conclusion: In our study, the vast majority (76.7%) of the patients acquired their bloodstream infection in the hospital. The patients

with BSIs caused by ESBL-producing *E. coli* and *K. pneumoniae* strains were more likely to have had longer hospitalisations and ICU stay prior to infection, and to have higher APACHE II score than were the patients with BSIs caused by non-ESBL-producing. The invasive procedures, neutropenia, and previous antibiotic therapy were more common among ESBL-producing group than they were among non-ESBL group. Bacteraemia caused by ESBL-producing strains is associated with higher mortality rate (19.7%) than the non-ESBL group (9.2%).

Table l. Distribution of personal and clinical characteristics of patients with bloodstream infection caused by ESBL-producing and non-ESBL-producing strains of *Escherichia coli* and *Klebsiella pneumoniae*.

Characteristic	ESBL group (n = 61; %)	Non-ESBL group (n = 98; %)	*P*-value
Nosocomial onset of infection [no (%) of subjects]	52 (85.2)	70 (71.4)	0.045
Age, mean±SD (range)	62.23±15.48 (17–83)	60.48±17.65 (17–90)	0.49
Sex (male/female)	38/23 (62.3/37.7)	50/48 (51/49)	0.164
No. with *K. pneumoniae*/*E. coli*	13/48 (21.3/78.7)	28/70 (28.6/71.4)	0.309
Median length of hospital stay (days)	11.48	7.06	0.03
Status or underlying illness [no (%) of subjects]			
Neurological diseases	7 (11.5)	11 (11.2)	0.961
Cardiovascular systemic diseases	6 (9.8)	14 (14.3)	0.411
Liver disease	2 (3.3)	9 (9.2)	0.206
Pancreatobiliary tract disease	0	5 (5.1)	0.075
Renal disease	17 (27.8)	20 (20.4)	0.190
Autoimmune disease	0	2 (2)	0.524
Haematological malignancy	9 (14.7)	9 (9.2)	0.281
Solid tumour	0	1 (1)	0.116
Solid organ transplant	17 (27.8)	17 (17.3)	1.0
Diabetes mellitus	9 (14.7)	11 (11.2)	0.326
Corticosteroid use	0	2 (2)	0.524
Immunosuppressive agent treatment	7 (11.5)	5 (5.1)	0.139
Any surgical procedure and trauma	19 (31.1)	19 (19.4)	0.091
Invasive procedures[a]	40 (65.6)	24 (24.5)	<0.001
Neutropenia (<500/mm^3)	1 (1.6)	12 (12.2)	0.018
Previous antibiotic therapy	28 (46)	31 (31.6)	0.07
Primary site of bacteraemia [no (%) of subjects]			
Lower Respiratory Tract	5 (8.2)	3 (3.1)	0.150
Urinary tract	28 (46)	40 (41)	0.448
Skin or soft tissue	11 (18)	13 (13.3)	0.414
Liver-Pancreatobiliary tract	5 (8.2)	11 (11.2)	0.537
Unknown	11 (18)	30 (31)	0.078
APACHE II score, mean±SD (range)	15.5±6.4 (3–33)	9.8±4.84 (0–21)	<0.001
ICU stay at time of initial bacteraemia	5.23	2.37	0.013

[a] Invasive procedures: insertion of catheter (e.g. a central venous catheter and urinary catheter), endoscopic retrograde cholangiopancreatography, insertion of nasogastric tube, parenteral nutrition.

Table 2. Clinical outcomes for patients with BSIs caused by ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates versus those for patients with BSIs caused by non-ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates

Outcome	No. of patients with the indicated outcome (%)		
	ESBL group (n = 61; %)	Non-ESBL group (n = 98; %)	*P*
Clinical			
Complete response	43 (70.5)	90 (91.8)	<0.001
Failure	18 (29.5)	3 (3.1)	<0.001
Uncertain		5 (5.1)	
Death within 30 days	12 (19.7)	9 (9.2)	0.05[a]

[a] OR (95% CI), 2.4 (0.95–6.15).

P2085 *Salmonella enterica* producing extended-spectrum β-lactamases in Zaragoza, Spain, 2001 to 2005

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Objective: To study the prevalence of *Salmonella enterica* producing extended-spectrum betalactamases (ESBL) in the sanitary area of the Hospital “Clinico Universitario Lozano Blesa”, Zaragoza, Spain.

Methods: From the cases of gastroenteritis due to *Salmonella enterica* detected in our area from 2001 to 2005, we selected the isolates with reduced susceptibility to extended spectrum cephalosporins (ESC) using the Wider® semi-automatic system for identification and MIC-determination. Complete serotyping was performed according to the Kauffmann-White scheme. The ESBL phenotype was detected by the double disk synergy test and by E-test. For isolates showing an ESBL phenotype, the genes of CTX-M, SHV and TEM groups were detected by PCR and sequencing. Detection of the gene cassettes of class 1 integrons were also performed by PCR. Transfer of ESC-resistance was performed by conjugation on solid medium using *Escherichia coli* K12J5 resistant to sodium azide. Plasmid analysis was performed by the S1-nuclease method. Plasmids were typed for their incompatibility group by a PCR-based replicon typing method. The genetic diversity of the *S. enterica* isolates was assessed by a pulsed field electrophoresis following the PulseNet standardised protocol.

Results: We collected 2170 *Salmonella enterica* isolates from 2001 to 2005. Only five ESBL-producing isolates were detected (0.23%) in five outpatients. Four serotype Virchow isolates produced CTX-M-9. They showed a reduced susceptibility to ceftriaxone (MIC=32 mg/L) but were susceptible in vitro to ceftazidime (MIC=1 mg/L). They were resistant to aminoglycosides (kanamycin, streptomycin and spectinomycin), tetracycline, trimethoprim and sulfonamides. A single class 1 integron gene cassette was found (dfrA16-aadA2), identical to that of complex integron In60. A 330kb-conjugative plasmid belonging to the incompatibility group Inc HI2 was found in all the tranconjugants, co-transferring all the resistances seen in parental isolates. The fifth isolate belonged to serotype Enteritidis containing a blaCTX-M-1 gene located on a 290kb-conjugative plasmid of Inc I1 group. Two PFGE profiles sharing 90% of identity were seen in the ESC-resistant Virchow isolates.

Conclusion: These are the first cases of ESBL-producing *Salmonella* in our area and seem to be related with other community-acquired isolates described in Spain and France. The increasing presence of CTX-M genes among Enterobacteriaceae is probably due to the conjugative plasmid location.

P2086 Enterobacteriaceae producing extended-spectrum β-lactamase: report on the first nine months notifications according to the Swedish Communicable Disease Act

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Objectives: In Sweden, Enterobacteriaceae producing Extended Spectrum Beta-Lactamases (ESBL) are mandatory notifiable to the authorities since February 1, 2007. Several local outbreaks of ESBL-producing bacteria, as well as a governmental bill aiming at decreasing healthcare related infections, have urged these notifications. Reporting of bacteria with ESBL is based on laboratory notifications only.

Methods: Analysis of all laboratory notifications posted in the national web-based surveillance system SmiNet2, 2007–02–01 to 2007–10–31. Information on age, gender and cultured material was available, but not information on reason for sampling (screening vs. clinical) and place for acquisition (imported vs. domestic, community vs. hospital).

Results: A total of 1648 patients were notified during the period. Reports came from all 21 counties of Sweden, corresponding to an average national incidence of 18.0 per 100,000 inhabitants. The most commonly reported species was *E. coli*, accounting for 72% of all cases, followed by *K. pneumoniae* with 12%. Information concerning species was missing for 13% of the cases. Bacteria were cultured from urine in 73% of the cases. The second most common source for ESBL-producing Enterobacteriaceae was faecal samples. Eighty cases grew bacteria in the blood and one case in cerebrospinal fluid. The distribution of gender and age differed depending on species. Among ESBL-producing *E. coli*, 69% were derived from women with a mean age of 54.5 years. This was almost seven years less than the corresponding mean age of men. The *K. pneumoniae* ESBL cases were almost equally distributed between sexes, but with higher mean ages (68 years for women and 65.5 for men).

Conclusion: The first nine months of mandatory notification show that ESBL-producing bacteria have become a nation-wide problem in Sweden. An endemic situation seems to have developed, possibly putting patients at risk when receiving empiric antibiotic treatment. In order to meet the increasing ESBL prevalence in Sweden, an action programme has been suggested by an expert group from authorities and health professionals.

P2087 The efficacy of tigecycline against AmpC producing *Enterobacter* spp. and *Serratia marcescens*

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Objectives: Beta-lactamase production is the most common cause of β-lactam antibiotic resistance in Gram-negative bacteria. AmpC β-lactamases, presumably chromosomally mediated, are associated with multi-drug resistance in both *Enterobacter* spp. and *Serratia* spp. The emergence of these difficult to treat strains has severely reduced therapeutic options and resulted in treatment failures. Tigecycline, a new glycylcycline, offers the potential of enhanced activity against AmpC producing strains. The tigecycline evaluation surveillance trial (T.E.S.T.) evaluated the activity of tigecycline and comparators against AmpC producing *Serratia marcescens* and *Enterobacter* spp. from a global population.
Methods: 862 clinically significant isolates with the AmpC phenotype were collected between 2004 and 2007. MICs were determined at each site using common broth microdilution panels and results interpreted as specified by CLSI at each site. Additional testing was performed by Laboratories International for Microbiology Studies (LIMS), a subsidiary of International Health Management Associates, Inc. (IHMA, Schaumburg, IL, USA).
Results: MIC50/90 (μg/mL) and % susceptible (S) of tigecycline and comparators are shown in the table.

Drug	*Enterobacter* spp. (n = 821)			*Serratia marcescens* (n = 41)		
	%S	MIC_{50}	MIC_{90}	%S	MIC_{50}	MIC_{90}
Tigecycline	87.6	0.5	4	90.2	1	2
Amikacin	95.5	2	8	85.4	4	32
AmoxClav	0	>32	>32	0	>32	>32
Cefepime	100	4	8	100	4	8
Ceftazidime	0	>32	>32	0	>32	>32
Ceftriaxone	0	>64	>64	0	64	>64
Imipenem	99.6	0.5	1	100	1	1

Conclusions: Tigecycline is a promising expanded broad spectrum antibiotic showing significant activity against AmpC producing *Enterobacter* spp. and *Serratia marcescens* from a world wide population. With MIC50 values of 0.5 μg/mL versus *Enterobacter* and 1 μg/mL versus *Serratia*, this study validates tigecycline's potent inhibitory activity against these pathogens.

P2088 Emergence of fluoroquinolone-resistant *Shigella flexneri* type 2a from diarrhoea patients in Kolkata, India

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Objectives: Shigellosis is a major cause of diarrhoeal related morbidity and mortality, especially in the developing world. Diarrhoea caused by *Shigella* can usually be treated effectively by antimicrobial therapy. However, the emergence and dissemination of multidrug-resistant strains of *Shigella* is now an increasing global health problem that is complicating the therapeutic management of cases of shigellosis. Recently, fluoroquinolone resiatant *Shigella flexneri* type 2a strains, the predominant serotype have emerged in Kolkata, India and found to be multiple drug resistant (MDR). The aim of this study was to characterize MDR *Shigella flexneri* type 2a strains using phenotypic and genotypic methods.
Methods: Thirty one representative strains of *Shigella flexneri* 2a, isolatedduring 2004–2006 from children suffering from diarrhoea were examined in this study. *Shigella* species recovered were serotyped by a slide agglutination test with commercially available antiserum (Denka Seiken Co. LTD, Tokyo, Japan). Antimicrobial susceptibility pattern, detection of *Shigella* enterotoxin genes (set1), and virulence genes, analysis of plasmid DNA, and Pulse Field Gel Electrophoresis were performed following standard protocol.
Results: All the isolates tested, exhibited uniform resistance pattern however all were sensitive to ceftriaxone and azithromycin. All the isolates showed identical plasmid profile (3.03, 3.9 & 54.24 Kb).PFGE analysis of XbaI-digested chromosomal DNA of the fluroquinolone resistant *S. flexneri* serotype 2a strains yielded 15 to 18 reproducible DNA fragments ranging in size from approximately 20 to 388 kb. On the contrary the fluroquinolone-sensitive strains yielded 18 to 20 reproducible DNA fragments ranging in size from approximately 20 to 485 kb.
Conclusion: Multidrug resistance was common among the *S. flexneri* 2a strains. These results show that the multidrug resistance among serotype 2a strains could be conferred by plasmids. Fluroquinolone-resistant isolates showed more genetic homology, in contrast to fluroquinolone-sensitive strains. Interestingly an overall analysis of the PFGE results conclude that fluroquinolone sensitive serotype 2a originating from the different ancestral clones was found previously in Kolkata whereas the occurrence of fluroquinolone resistant isolates with homogeneous PFGE pattern in recent times confers that strains originating from a single clone are circulating in the environment, which was not found before.

P2089 High rates of plasmid-mediated quinolone resistance QnrB variants among ciprofloxacin-resistant *Escherichia coli* and *Klebsiella pneumoniae* from urinary tract infections in Korea

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Objectives: The aims of this study were to investigate the prevalence of qnrA, qnrB, and qnrS determinants and their molecular characteristics in ciprofloxacin-resistant isolates of *Escherichia coli* and *Klebsiella pneumoniae* from urinary tract infections in Korea.
Methods: A total of 202 non-duplicated clinical isolates of ciprofloxacin-resistant *E. coli* (N=143) and *K. pneumoniae* (N=59) were collected from urinary tract infections between July 2005 and Aug. 2006. The qnr determinant screening was carried out by PCR amplification of qnrA, qnrB, and qnrS; and all positive results were confirmed by direct sequencing of the PCR products. For qnr-positive strains and their conjugants, antimicrobial susceptibility tests and pulsed-field gel electrophoresis were performed.
Results: The qnrB gene was detected in 41 of the 202 isolates. Among 33 of 59 (55.9%) *K. pneumoniae* isolates showing qnrB, 29 isolates contained the qnrB4 gene, 3 isolates had the qnrB2 gene, and 1 isolate had the qnrB6 gene. All 8 (5.6%) of the qnrB-positive isolates among the 143 *E. coli* strains possessed the qnrB4 gene. The MICs of ciprofloxacin for the transconjugants were 0.03 to 2 ug/ml, representing an increase of 4 to 256 fold relative to the recipient, *E. coli* J53Azr. Resistances to various other antimicrobial agents also were transferred with the plasmid. The PFGE analysis revealed indistinguishable or closely related patterns in several strains, and highly diverse patterns in general.
Conclusions: qnrB variants, especially the qnrB4 subtype, are highly prevalent in ciprofloxacin-resistant *E. coli* and *K. pneumoniae* from urinary tract infections in Korea. The emergence of plasmid-mediated quinolone resistance may contribute by several means to the rapid increase in bacterial resistance to these drugs. Thus, we are paying close attention to the course of plasmid-mediated qnr resistance in various clinical specimens of Enterobacteriaceae.

P2090 Epidemiology of a new population of quinolone-resistant *Salmonella enterica*

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Objectives: In 2003, we described a new quinolone resistance phenomenon in *Salmonella enterica* isolates from Southeast Asia. These

isolates show reduced ciprofloxacin susceptibility but are susceptible or only low-level resistant to nalidixic acid. The purpose of the present work was to survey the epidemiology of this new resistance phenotype.
Methods: In this study, 599 *Salmonella enterica* isolates collected between 2002 and 2007 from Finnish travellers returning from abroad were analysed and the results were compared with 829 *Salmonella* isolates collected between 1995 and 2001. Annually the first 100 foreign *Salmonella* isolates were collected. The MICs of the isolates to ciprofloxacin and nalidixic acid were determined by the standard agar dilution method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. The susceptibility data were analysed by using the WHONET 5.4 computer programme.
Results: From 1995 to 2002, all *Salmonella* isolates showing reduced ciprofloxacin susceptibility were uniformly resistant to nalidixic acid. From 2003 to 2007, 9 (9.0%), 8 (8.1%), 2 (2.0%), 5 (5.0%) and 13 (13.0%) of the tested isolates, respectively, showed reduced ciprofloxacin susceptibility (MIC $\geqslant$0.125 mg/L) but were either susceptible (MIC < 32 mg/L) or low-level resistant (MIC=32 mg/L) to nalidixic acid. Epidemiological studies showed that all isolates showing the new resistance phenotype were from Southeast Asia, whereas the isolates exhibiting reduced ciprofloxacin susceptibility and nalidixic acid MIC > 32 mg/L were from different origins, 50% of them being from Southeast Asia. Among the new resistance phenotype, S. Corvallis and S. Stanley were the most prevalent serotypes, whereas among the nalidixic acid resistant isolates, S. Enteritidis and S. Virchow were the most prevalent.
Conclusion: Our study revealed that the new phenotype of quinolone-resistant *Salmonella enterica* is increasing. Moreover, this phenotype was found in several serotypes being, thus, not restricted to one clone only. Although these strains are clustered in Southeast Asia, they are of concern since the amount of travellers is continuously increasing in that part of the world.

P2091 First detection of the plasmid-mediated quinolone resistance in extended-spectrum β-lactamases producing Enterobacteriaceae isolated from in-and out-patients in Switzerland

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Objective: The aim of the present study was to determine the prevalence of plasmid-mediated quinolone resistance qnr genes in ESBL-producing enterobacteriaceae strains isolated in a private laboratory.
Methods: Identification and antimicrobial susceptibility testing were performed using the VITEK 2 system (bioMérieux SA). PCR detection for plasmid-mediated quinolone resistance was performed using primers amplifying intragenic fragments of qnrA, qnrB and qnrS. MICs of nalidixic acid and fluoroquinolones were determined for the qnr-positive isolates using the Etest method. ESBL characterisation was performed for qnr-positive isolates by PCR of the TEM, SHV and CTX-M genes.
Results: 156 non-duplicate ESBL-producing enterobacteriaceae isolated from April 2001 to February 2006 were analysed. They provided from 155 patients from whom 87 (56.1%) were outpatients and 68 (43.9%) in-patients. The sex ratio of patients was 112/43 (female/male). Bacterial specie distributed as follows: *Escherichia coli* (n = 125, 80%), *Klebsiella pneumoniae* (n = 23, 14%), *Citrobacter freundii* (n = 2), *Klebsiella oxytoca* (n = 2), *Enterobacter cloacae* (n = 1), *Morganella morganii* (n = 1), *Proteus penneri* (n = 1) and *Salmonella enterica* (n = 1). The global prevalence of qnr-positive isolates was 3.2% with five isolates harbouring a qnrB gene: two isolates of *C. freundii* with the qnrB4 allele, 2 isolates of *K. pneumoniae* with the qnrB4 allele and one isolate of *E. coli* with the qnrB2 allele. None of the isolates harboured qnrA or qnrS genes. These five isolates were coming from urines of four different patients of whom three were followed by private practitioners in the community setting. Four isolates presented a high level resistance to fluoroquinolones (MIC > 32 mg/L) and one *C. freundii* isolate showed a intermediate level of fluoroquinolone resistance (fluoroquinolone MIC below 8 mg/L). The main type of ESBL of qnr-positive isolates was CTX-M-15 (n = 3), followed by CTX-M-3 (n = 1) and CTX-M-9 (n = 1).
Conclusion: This is the first report of plasmid-mediated quinolone resistance from clinical isolates in Switzerland, in the hospital as well as in the community setting. Although a low prevalence of qnr determinants was observed among ESBL-producing enterobacteriaceae, dissemination of qnrB genes is already present in Switzerland.

P2092 Prevalence of antimicrobial resistance and detection of qnr in avian Salmonellae in two EU regions, 1998–2006

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Objectives: In many countries, nontyphoidal *Salmonella* is a main cause of food-borne illness. Antimicrobial treatment is recommended for severe salmonellosis, with fluoroquinolones (FQs) as drugs of choice. As poultry is a major source of *Salmonella* to infect humans, a 9-year survey of FQ susceptibility was conducted. As plasmid-mediated quinolone resistance has recently been detected in *Salmonella*, we evaluated the isolates with reduced susceptibility to ciprofloxacin (CP) for qnr gene prevalence.
Methods: In the first programme, caecal samples from chickens were randomly taken at a Belgian abattoir. In the second, carcass samples were collected from chickens and turkeys processed in various abattoirs across Germany. Susceptibilities to CP and nalidixic acid (NA) and non-FQs such as ampicillin (AM), cefotaxime (CT), chloramphenicol (CA), gentamicin (GM), streptomycin (S), tetracycline (TE) and trimethoprim/sulfadiazine (TS) were assessed by agar dilution. Resistance was calculated using CLSI breakpoints (M100-S16). The presence of qnrA, qnrB and qnrS was tested by PCR and sequence techniques.
Results: In all, 4792 *Salmonella* isolates (Belgium 408; Germany 4384) comprising 53 serotypes were recovered. In both surveys, clinical resistance to CP was absent in all but one of the isolates tested (MIC 4 mg/l). Reduced CP susceptibility (MICs 0.12–2 mg/l) was 32% and 11%. S. Hadar and S. Saintpaul were the most frequent serotypes exhibiting reduced CP susceptibility, which virtually resembles the resistance to NA. CT resistance was 1.7 and 0%, respectively, whereas reduced CT susceptibility (MICs 1–32 mg/l) amounted to 4.3 and 0.8%. In contrast, resistance to AM, CA, S, TE and TS amounted to 46, 9, 29, 34 and 20% in Belgium and 21, 5, 11, 14 and 16% in Germany. GM resistance did not exceed 1% in either programme. Of 688 isolates tested for qnr genes, 4 isolates (0.6%) carried qnrS (S. Give, S. Infantis, S. Saintpaul) or qnrB (S. Derby). These 4 isolates were isolated in 2003, 2004 and twice in 2005.
Conclusions: Clinical resistance among *Salmonella* from chickens and turkeys to CP or CT, particularly important drugs for treating salmonellosis in humans, was zero to very low. However, for most older drugs notably higher rates were assessed. Reduced susceptibility to CP and CT varied markedly for CP with the different serotypes. The findings also indicate that the occurrence of qnr genes seems to be uncommon in avian *Salmonella* within the EU regions.

P2093 Decreasing susceptibility to ciprofloxacin in foreign travel-related cases of enteric fever in England, Wales and Northern Ireland: results from an enhanced surveillance pilot

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Objectives: The Laboratory of Enteric Pathogens (LEP) of the Health Protection Agency of England, Wales, and Northern Ireland (EWNI) is the reference centre for *Salmonella enterica* serovars Typhi (ST) and Paratyphi A (SPA) for the United Kingdom (UK) and receives isolates from all cases of infection in the UK for confirmation and antibiotic susceptibility testing. Since 2001, LEP has reported on an increase in the number of isolates of both ST and SPA that have exhibited decreased susceptibility to ciprofloxacin [MIC: 0.25–1.0 mg/L] (CpI), the first line drug of choice for treatment of enteric fever for most physicians in the UK. Travel history information has, however, been lacking for most cases. To learn more about enteric fever reported in EWNI and improve

travel history reporting, a pilot study of enhanced surveillance of cases began 1 May 2006.

Method: An enhanced questionnaire was completed for each laboratory-confirmed and typed case of enteric fever as part of routine follow up according to UK guidelines. The information was collected in a MS Access database and cases known to have been acquired abroad with onset dates between 1 May 2006 and 30 April 2007 were extracted and analysed using MS Excel.

Results: During the report period, 373 cases of enteric fever (176 ST, 193 SPA, and four SPB) that were acquired abroad were reported. Seventy-two percent of SPA isolates and 75% of ST isolates were CpI, compared to 35% of ST isolates and 23% of SPA isolates reported by the LEP in 2001. Country of travel was available for 99% (369/373) of cases acquired abroad. For paratyphoid A: 93% (100/107) of cases acquired in India, 32% (21/65) of those acquired in Pakistan, and 100% (13/13) of those acquired in Bangladesh had isolates of SPA with CpI. For typhoid: 95% (72/76) of cases acquired in India, 62% (33/53) of those acquired in Pakistan, and 88% (23/26) of those acquired in Bangladesh had isolates of ST with CpI. Three further cases acquired in China (two) and Indonesia (one) also had isolates of SPA with CpI.

Conclusion: Strains of ST and SPA with decreased susceptibility to ciprofloxacin are increasingly being seen in cases that have arrived in the UK from the Indian sub-continent, in particular from India and Bangladesh. These results indicate that the use of ciprofloxacin may become increasingly limited as a first line treatment and that available alternatives should be considered, particularly in cases returning from India or Bangladesh.

P2094 Comparative in vitro activities of tigecycline and 11 other antimicrobial agents against 217 epidemiologically defined *Acinetobacter* bloodstream isolates

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Objectives: To evaluate the vitro activity of tigecycline and other antimicrobial agents against epidemiologically well-defined *Acinetobacter* bloodstream isolates.

Methods: The in vitro activity of tigecycline and 11 other antimicrobial agents was determined for 217 epidemiologically unrelated isolates belonging to the *A. calcoaceticus-A. baumannii* complex. Isolates were recovered from bloodstream infection in the United States between 1995 and 2002 during a prospective surveillance study (SCOPE). Species identification was confirmed by ARDRA; RAPD-PCR and PFGE patterns were obtained to exclude isolates that were clonally related, i.e. only one isolate per strain type was included. MICs were determined using microbroth dilution. CLSI/NCCLS guidelines were used for interpretation.

Results: MIC50s and MIC90s (mg/L) are depicted in the table.

Resistance rates were lower in *Acinetobacter* genomic species 3 and 13TU than in *A. baumannii* strains.

Antimicrobial agent	Range (mg/L)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	Susceptible (%)	Resistant (%)
Tigecycline	⩽0.125–4	⩽0.125	0.5	Nd	Nd
Doxycycline	⩽0.25–>32	⩽0.25	4	95.4	4.1
Ampicillin/sulbactam	0.5/0.25–>32/16	4	32	79.3	12.9
Piperacillin	2–>64	16	>64	50.7	30.0
Cefepime	⩽0.25–>32	4	32	54.8	16.1
Imipenem	⩽0.25–32	0.5	2	95.4	1.4
Gentamicin	⩽0.25–>32	1	32	69.6	24.4
Tobramycin	⩽0.25–32	0.5	2	94.9	3.2
Amikacin	⩽1–128	2	8	98.2	0.9
Levofloxacin	⩽0.25–>32	⩽0.25	16	69.1	26.3
Colistin	⩽0.125–4	0.25	0.5	99.1	0.9
Rifampicin	1–>32	4	8	Nd	Nd

Conclusions: Tigecycline, amicacin, and colistin showed the highest in vitro activity against *A. baumannii* bloodstream isolates. Our data confirm the high resistance of clinical *A. baumannii* isolates against betalactams and quinolones but resistance to imipenem and aminoglycosides was less than suggested from surveillance studies that don't control for copy strains associated with endemic or outbreak situations.

P2095 Molecular characterisation of aminoglycoside resistance in Enterobacteriaceae

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Objectives: Bacterial resistance to antimicrobial agents has become an increasing problem in clinical practice, limiting the therapeutic options. Aminoglycoside resistance may be due to several mechanisms, among which aminoglycoside-modifying enzymes are particularly important.

We report here the prevalence of the aminoglycoside-modifying enzymes present in aminoglycoside-resistant Enterobacteriaceae isolated during the first three months of 2006.

Methods: The susceptibilities to aminoglycosides: amikacin, gentamicin, kanamycin, netilmycin, neomycin, spectinomycin, streptomycin and tobramycin were determined by disc diffusion method. The MICs of the aminoglycosides resistant isolates were determined by Etest. Detection and characterisation of the modifying-enzymes was done by PCR. Some of these amplicons were sequenced. ERIC-PCR or PFGE were used to study the clonal relationship between bacterial strains carrying the same aminoglycoside-modifying enzyme.

Results: During this period 803 isolates were recovered from 718 patients, 371 of which (46.2%) presented aminoglycoside resistance. Of the 8 aminoglycosides tested, the most affected was streptomycin (S) (342; 42.6%), followed by kanamycin (K) (103; 12.8%), gentamicin (G) (68; 8.4%), tobramycin (T) (64; 8%), neomycin (N) (62; 7.7%), spectinomycin (Sp) (36; 4.5%), netilmycin (Nt) (16; 2%) and amikacin (A) (6; 0.7%). Thirty-one clonally-related strains were excluded for enzyme characterisation.

The most frequent enzyme found was APH-3″-Ib (S) (61%), followed by ANT-3″-Ia (S,Sp) (40%), AAC-3-IIa (KGTNt) (10%), APH-3′-Ia (KN) (7.6%), AAC-6′-Ib (KTANt) (3.8%), ANT-2″-Ia (KGT) (3.2%) and AAC-6′-Ic (KTANt) (0.3%). Finally, the amplification results were negative for 37 strains.

Twenty-two percent of the strains presented more than one enzyme. ANT-2″-Ia and AAC-6′-Ic were always associated with other enzymes. The most frequent association was APH-3″-Ib + ANT-3″-Ia (48 strains) or this association with AAC-3-IIa (7) or APH-3′-Ia (6), and APH-3″-Ib + AAC-3-Iia (11).

Conclution: Extensive resistance was found within Enterobacteriaceae for streptomycin and also for kanamycin, tobramycin and netilmycin. The high number of strains with multiple aminoglycoside-modifying-enzymes is of particular concern.

P2096 In vitro susceptibility patterns to 13 antimicrobials against *Pseudomonas aeruginosa* cystic fibrosis patients in the UK

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Objectives: Respiratory tract colonisation and infection in cystic fibrosis (CF) patients contributes to the progressive decline in lung function. *Pseudomonas aeruginosa*, is the most common bacterial pathogen and is often exhibits multi-drug resistance. We conducted a surveillance of resistance to 13 commonly used agents amongst isolates from a large UK CF centre to provide an indicator of the prevalence of resistance in 2005–2007.

Methods: 478 strains of *P. aeruginosa* were isolated from CF sputum over a 24 month. Strains were identified by growth on *Pseudomonas* Isolation agar, API 20NE and confirmed by specific PCR. Isolates were classified as mucoid or non-mucoid and tested for susceptibility to amikacin, ceftazidime, ciprofloxacin, gentamicin, imipenem, meropenem, piperacillin/tazobactam, azlocillin, aztreonam, colistin, tobramycin, cefpirome and ticarcillin/clavulanate by the BSAC disc diffusion method.

Results: Only 21.2% of the isolates were sensitive to all antibiotics tested. The most active agent was colistin (1.1%) followed by tobramycin (13.4%) and piperacillin/tazobactam (17.8%).11 isolates were resistant to all antibiotics except for colistin. Resistance to cefpirome was (74%), ciprofloxacin (40.5%), gentamicin (39.3%), ticarcillin/clavulanate (38.5%), imipenem (38.5%), meropenem (33.3%), aztreonam (31.6%), azlocillin (28.7%), ceftazidime (26.2%) and amikacin (25.3%). In-vitro susceptibility of mucoid *P. aeruginosa* isolates was greater than non-mucoid isolates ($p < 0.05$ for gentamicin, tobramycin, amikacin, ticarcillin/clavulante, azlocillin, aztreonam and ciprofloxacin). Multi-drug resistance ($\geqslant$5 antibiotics) occurred in 55.3% of isolates.
Conclusion: *P. aeruginosa* CF isolates exhibit very high rates of antimicrobial resistance. This not only complicates treatment of chronic infection and exacerbations difficult but may be an important reservoir of antimicrobial resistance determinants for dissemination to non-CF strains. Continued surveillance of antibiograms and monitoring for new resistance genes in this population is warranted.

P2097 Antibiotic resistance in Enterobacteriacea in Austrian intensive care units

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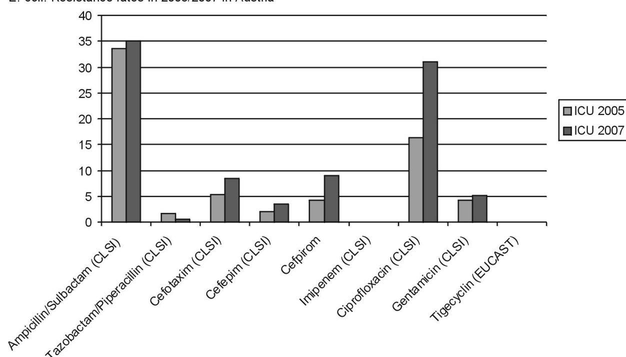
Background: Infections with resistant Gram-negative (GN) bacteria in intensive care settings have become a threat to modern medicine in recent years. Increasing resistance against first line drugs such as fluoroquinolones (FQ) or 3rd generation cephalosporines (3C), the appearance of metallo-betalactamase resistance (MBL) in some countries e.g. Greece and Great Britain and the lack of new drug developments with activity against GN pathogens are the main factors for increasing complexity of patient management. In the present study we analysed the current situation and time trends of in vitro activity of vital substances in intensive care units (ICUs) of 33 hospitals in Austria.
Methods: Susceptibility to 9 antibiotics of 1.125 consecutive enterobacteriacea strains isolated in clinical specimen in ICUs all over Austria during the periods January to May 2005 and January to May 2007 was determined at the National Reference Center for Nosocomial Infections and Antibiotic Resistance in Linz. Besides that data on patient characteristics, epidemiology and culturing practice were collected to set resistance prevalence in relation to factors of patient characteristics and clinical activity. Data from the European surveillance network EARSS has been used for comparison and to bring the findings in a larger context.



Results: For the most frequent GN pathogen *E. coli* (eco) rising resistance for FQ, 3C, and 4th generation Cephalosporins (4C) as well as an increase of ESBL-producing strains were found in Austrian ICUs. In contrast to that, the activity of Carbapenems (C) as well as the activity of the recently introduced drug tigecycline (T) was still very high. In *Klebsiella* species (ksp) no rising trend in resistance for FQ, 3C, 4C and rates of ESBL-producing strains were detected. This is in accordance with findings in EARSS where resistance rates of FQ and 3C for invasive eco isolates has also risen substantially (FQ: 7% to 27%; 3C: 0% to 8%) between 2001 and 2007. Resistance rates for FQ (12%/12%), 3C (6%/5%) and C (0%/0%) for *K. pneumoniae* (kpn) have on the other hand remained stable since the beginning of the EARSS data collection in 2005.
Conclusions: There is still in vitro activity of a number of substances especially the reserve drugs C and T against eco and kpn/ksp. It is surprising that typical nosocomial pathogens such as ksp and kpn show no change in resistance over time unlike eco which shows an enormous increase in resistance against firstline therapeutic options.

P2098 Molecular typing, serotyping and antibiotic resistance patterns of *Salmonella enterica* strains isolated from foods and humans in Valencia, Spain

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Aims: Salmonellosis is the most common foodborne disease worldwide, being human and animal excreta the most important sources of *Salmonella* in environment. S. Enteriditis and S. Typhimurium are two of the most prevalent serotypes transmitted from animals to humans. The emergence of *Salmonella* strains with resistance to fluoroquinolones and third-generation cephalosporins is a public health problem, which results in limitation and even failures of the effective treatment. This drug-resistant *Salmonella* emergence is mainly attributed to over use of antimicrobials in food animals.
The aim of this work consisted in evaluating the prevalence of antibiotic resistances in *Salmonella* strains isolated from different sources in our geographical area, and investigating serovars and genotypes distribution among the isolates.
Methods: A total of 200 *Salmonella* strains were isolated from water, foods (mainly chicken meat), poultry faeces and human clinical samples. Serotypes, antibiotic resistance patterns and genotypes were investigated. Resistance to 12 antibiotics was determined by Disk diffusion tests (NCCLS guidelines). Molecular typing was performed by PFGE with XbaI enzyme and AFLP techniques.
Results: A total of 25 different serotypes were identified. S. Enteritidis was the predominant serotype (26.3%) followed by Typhimurium (18%) and Hadar (13%). All the strains were susceptible to Ceftriaxone. A high proportion (86%) of the isolates was resistant to at least one antibiotic, being 31% multidrug-resistant. Serotypes Typhimurium and Hadar presented a high rate of multiresistant strains. 34% of the isolates, most of them from poultry faeces, were quinolone-resistant. PFGE allowed for clustering strains with similar origin or serotype. AFLP technique was highly discriminatory, being all the patterns serotype-specific. However, neither by PFGE, nor by AFLP it was possible to correlate genotypes with resistance patterns.
Conclusions: A high prevalence of antibiotic resistant strains were found among *Salmonella enterica* strains, most of them from animal food and poultry faeces, confirming the role of veterinary use of antimicrobial agents in the emergence of this public health problem. Molecular typing techniques AFLP and PFGE are useful epidemiological tools for subtyping *Salmonella enterica* strains.
Acknowledgements: This work was supported by Ministerio de Educación y Ciencia, Spain (Project AGL2005–07776-C03–01, national and FEDER funds).

P2099 Antimicrobial resistance mechanisms in clinical isolates of *Pseudomonas aeruginosa*

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Objectives: *Pseudomonas aeruginosa* is a significant nosocomial pathogen innately resistant to a wide variety of antimicrobials. It is an opportunistic pathogen capable of causing both local and systemic infections.
This study was undertaken to examine the antimicrobial resistance mechanisms utilised by clinical isolates of *P. aeruginosa*.

Methods: *Pseudomonas aeruginosa* isolates resistant to fluoroquinolones, β-lactams, carbapenems and aminoglycoside were collected from St. James's Hospital Dublin.
Antibiograms were determined by the disk diffusion method according to CLSI guidelines. The presence of fluoroquinolone resistance-associated mutations in gyrA, gyrB and parC was determined by PCR and sequencing. Genes encoding aminoglycoside-modifying enzymes were detected using PCR and sequencing. ESBL detection was carried out using the combined disk test. The presence of β-lactamase enzymes was determined by nitrocefin tests. Isolates were investigated for the presence of metallo-β-lactamases (MBL) using MBL Etest strips according to manufacturer's guidelines
The clonality of the isolates was determined by Pulsed Field Gel Electrophoresis (PFGE) using the Xba1 enzyme.
Results: 75% of the fluoroquinolone resistant isolates sequenced possessed a substitution of Ile for Thr-83 in GyrA. This result is in accordance with previous studies. One isolate possessed the less common mutation of Asn for Asp-87 in GyrA. No isolates had a mutation at position 106 in GyrA. No mutations were found in GyrB. The substitution of Leu for Ser-87 in ParC was found in 67% of isolates sequenced. An infrequent mutation of Gln for Glu-91 in ParC was found in two isolates. One isolate was positive for the aminoglycoside-modifying enzyme aac(6′)-Ib.
No ESBLs were detected. Eleven isolates tested positive using nitrocefin test indicating hyper-production of AmpC. Although MBLs are associated with high level carbapenem resistance, six isolates with MIC's ranging from 16–32g/L were positive according to imipenem Etest.
The PFGE patterns did not suggest the predominance of a single clonal genotype within the population.
Conclusion: Common and rare chromosomal mutations, associated with fluoroquinolone resistance, were detected. A high percentage of isolates have a derepressed AmpC enzyme and preliminary results suggest the presence of MBLs. This is a worrying trend. Continued reporting along with antibiotic stewardship are required to prevent the emergence and spread of resistant isolates.

P2100 *P. aeruginosa* with hypermutable phenotype in cystic fibrosis patients

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Background: Hypermutable phenotype (HPM) strains are those that have an increased (up to 1,000-fold) spontaneous mutation rate due to defects in genes involved in DNA repair or error avoidance systems. The presence, in cystic fibrosis (CF) patients, of a high proportion of HPM (or mutator) *P. aeruginosa* (PA) strains, was documented (Oliver et al. 2000). The acquisition of a stable mutator phenotype may confer a selective advantage for PA in the pulmonary environment in CF, because both high mutation rates and increased promiscuous recombination allow faster adaptation. Aim. Determine occurrence and antibiotic resistance PA HMPs in Italian CF pts.
Methods: 154 non-mucoid, 100 mucoid and 18 SCV non-multiresistant PA from 170 pts from 4 Italian CF Centres were studied. HMP was determined as previously described (Macia et al. 2004) by disk-diffusion and Etest for 10 antibiotic; results were read after 24 h at 35ºC; plates were re-incubated for further 12 h and HMPs were defined as strains showing a sub-population grown after 36 h and resistant to 3 o more antibiotics. A preliminary comparison between the reference mutation frequencies measurement test (Oliver et al. 2000) was performed on 10 PA strains and PA01, to assess the laboratory agreement with the disk-diffusion test for hypermutability. The genetic relationship of all PA was determined by BOX-PCR.
Results: Our results showed that Macia's test and the reference mutation frequencies measurement test overlap in assessing HMP strains. 32% of pts was colonised with HMPs, mean age 28 yrs; pts without HMPs showed a mean age of 17 yrs. HMPs distribution was 17% in non-mucoid, 28% in mucoid and 39% in SCV strains. No correlation with HMP occurrence and shared genotypes was found. The % of antibiotic resistance in HMPs was AK 33, TO 12, CL9, FEP 51, CAZ 41, MEM 26, P/T42, T/C 47, CIP 35 and LEV 39.
Conclusion: The occurrence of 32% found, confirm previous rates reported from CF pts in other country (Oliver et al. 2000). The high occurrence found of in mucoid (28%) and SCV (39%) strains, typical in long term chronic colonisation, stress the possible role of CF lung environment in inducing the expression of HMPs. In these strains the emerging resistance to TO and particularly to CL is worrisome for antimicrobial treatment implications.
This study was supported by the Italian Cystic Fibrosis Foundation Grant FFC#8/2005

P2101 Enterobacteriaceae in urinary tract infections: a laboratory view

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Objective: To find out the frequency an antimicrobial resistance on enterobacteriaceae in urinary tract infections.
Methods: All urine isolates from urine culture were collected from inpatients in university-affiliated Hospital of Infectious Diseases – Iasi in 2007. The analysis was based on the microbiology laboratory data according to the standard techniques.
Results: A total of 736 isolates from urine culture were analysed. There were 79.5% enterobacteriaceae. The predominant species was *E. coli* (77.8%), followed by *Klebsiella* spp. (13.7%) with predominant species *K. pneumoniae* (88.8%). The other pathogens were: *Enterobacter* (4.9%), *Proteus* (2.7%), *Citrobacter* (1%), *Morganella* spp. and *Hafnia* (<1%). The highest rate of resistance of *E. coli* among the different antimicrobials classes was: amoxicillin-clavulanate (17%), ceftazidime and cefoperazone (27.1%), ceftriaxone (23.4%), cefotaxime (15.4%), cefoperazone-sulbactam (4.6%), piperacillin-tazobactam (7.5%), imipenem (1.1%), ertapenem (0.7%), ofloxacin (29%), ciprofloxacin (25%), tobramicine (37.8%), amikacine (20%), colistin (8.8%); susceptibility to meropenem is 100%. The most important resistance for *Klebsiella* were: amoxicillin-clavulanate (35.8%), ceftazidime (30%), cefoperazone-sulbactam (25.3%), ertapenem (16%), colistin (6.7%).
Conclusions: The most common pathogen among isolates from urine culture was *E. coli*. There is an increasing local resistance of enterobacteriaceae at available antimicrobial agents.

P2102 Antimicrobial susceptibility profiles of *Shigella* spp. and *Salmonella* spp. isolated from children <5 years of age with diarrhoea in southern Mozambique

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Objective: To assess the antimicrobial profiles of *Salmonella* spp. and *Shigella* spp. isolated in children <5 years of age with diarrhoea attended at the Manhiça Hospital District, in Southern Mozambique
Methods: Rectal swabs were systematically collected in children <5 years of age complaint with diarrhoea. 1351 rectal swabs were cultured for *Salmonella* and *Shigella* isolation. *Shigella* and *Salmonella* strains were characterised biochemically and confirmed by API20E (BioMerieux, Marcy-I'Etoile, France), and serotypes determined by slide agglutination using specific antisera. Antimicrobial susceptibility testing to ampicillin (Amp), chloramphenicol (Chl), cotrimoxazole (SXT), tetracycline (Tet), nalidixic acid (Nal), Ceftriaxone (Cro) was performed by disc diffusion method and interpretative category of susceptibility (susceptible, intermediate or fully resistant) was determined according to Clinical Laboratory Standard Institute (CLSI). Nalidixic acid and ampicillin resistant strains were tested to ciprofloxacin (Cip), and amoxicillin plus clavulanic acid (A/C), respectively. Multi-drug resistance was considered when the isolates presented full resistance to three or more non-related antimicrobial agents.

Results: One hundred eleven (8.2%) *Shigella* (being 95 *Shigella flexneri*, 15 *Shigella sonnei* and 1 *Shigella dysenteriae*) and 42 (3.1%) *Salmonella* (10 S. Infantis, 9 S. Typhimurium, 5 S. Virchow, 18 other *Salmonella* serotypes) were recovered.
High prevalence of antibiotic resistance was observed among *Shigella* strains (55% for Chl, and 84% for SXT). *S. flexneri* were more resistant to Amp (68% versus 27%, p = 0.003) and Chl (68% versus 0%, p < 0.0001) than *S. sonnei*, while *S. sonnei* were more resistant to tetracycline (93% versus 63%, p = 0.002). *S. flexneri* presented high level of MDR compared to others serotypes isolated in this study
Approximately 13% of resistance to Chl and 40% to Amp was found among *Salmonella* strains with S. Typhimurium more resistant than others. Among others serotypes of *Salmonella*, two out of three *Salmonella* Isangi strains were tested and showed high levels of resistance to Amp, Chl, Tet and A/C.
Conclusions: *Shigella* strains were mostly resistant to available, inexpensive antibiotics including MDR strains while *Salmonella* spp. presents lower levels of antimicrobial resistance. Although high level of *Shigella* resistance, nalidixic acid which is first line treatment of shigellosis in the country is still effective.

P2103 Resistance trends of *E. coli* from urinary tract infections in hospitalised patients and consumption of antimicrobials, 1998–2006

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Objectives: To find out the frequency of *E. coli* among urinary pathogens and to investigate the resistance trends of *E. coli* to antimicrobials and the consumption of antimicrobials in hospital in period 1998 to 2006.
Methods: We analysed all pathogens from urine samples from patients hospitalised at University Clinical Centre Maribor. Urine cultures were done according to standard techniques and susceptibility was determined according to NCCL standards by disc diffusion method.
Results: In 1998 and 2006, 2563 and 3005 pathogens from urine cultures were analysed. The frequency of Gram-negative bacteria increased (from 64.7% to 75.9%) and *E. coli* was the most common pathogen determined, representing 39.1% (N=1002) and 44.6% (N=1339) of all isolates.
The resistance rates of *E. coli* to some most often used antimicrobials in period 1998–2006 were as follows: to amoxicillin-clavulanate 15.4% and 13.3% (p-NS), to cephalotin 7.8% and 16.8% (p < 0.001), to cefuroxime 2.8% and 3.2% (p-NS), to ciprofloxacin 4.9% and 13.0% (p < 0.001), to gentamicin 2.3% and 5.3% (p < 0.001), to cotrimoxazole 15.0% and 26.1% (p < 0.001).
The consumption of some antimicrobial agents in our clinic in period 1998–2006 was as follows (in DDD/100 hospital days): amoxicillin-clavulanate 100006 and 140809, cefuroxime 43404 and 100356, gentamicin 15908 and 13300, ciprofloxacin 41592 and 106513.
Conclusion: *E. coli* remains the most frequently isolated pathogen. A considerable rise in the resistance rates of *E. coli* to cephalotin, ciprofloxacin, gentamicin and cotrimoxazole was detected. Amoxicillin-clavulanate was the only one with slightly improved susceptibility, although it was the most often prescribed antimicrobial agent to inpatients and outpatients too. In 2006 we found the resistance rates of *E. coli* lower than 10% only to cefuroxime and to gentamicin. ESBL-producing strains among *E. coli* were detected in 1.5%.
We believe that it is necessary to rationalize persciption of ciprofloxacin in our clinic in order to prevent a further increase of resistance. Knowledge of most frequent uropathogens and their antimicrobial resistance patterns is essential to provide clinically successful, cost effective antimicrobila therapy for urinary tract infections.

P2104 High levels of antimicrobial resistance of diarrhoegenic *Escherichia coli* in Peruvian infants

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Objective: The purpose of this study was to determine the prevalence and antimicrobial susceptibility of the diarrheogenic *Escherichia coli* in Peruvian children less than 1 year of age with and without diarrhoea.
Methods: This was a prospective longitudinal cohort of 1025 Peruvian infants followed from 2 to 10 months of age in low-socio-economic communities in Lima, Peru. Five *E. coli* colonies per patient were analysed for the presence of Enterotoxigenic (ETEC), Enteropathogenic (EPEC), Shiga toxin-producing (STEC), Enteroinvasive (EIEC), Enteroaggregative (EAEC), and Diffusely Adherent *E. coli* (DAEC), in a multiplex real time PCR system, and analysed for their antimicrobial susceptibility by disk diffusion to ampicillin (Amp), amoxicillin-clavulanic acid (AMC), cefotaxime (Ctx), ceftazidime (Caz), gentamicin (Gm), nalidixic acid (Nal), ciprofloxacin (Cip), tetracycline (Tc), chloramphenicol (Cm), cotrimoxazole (Sxt) and nitrofurantoin (Ntr).
Results: The prevalence of the diarrheogenic *E. coli* was 29% (161/557) in the diarrhoea group and 30% (58/195) in the control group without diarrhoea; the most common *E. coli* groups were EAEC (14% vs.18%), EPEC (7% vs. 7%), DAEC (4% vs. 3%) and ETEC (4% vs. 2%), respectively. Among diarrhoeal samples, the diarrhoeogenic *E. coli* as a group exhibited high levels of antimicrobial resistance to Amp (85%), Sxt (79%), Tc (65%), Nal (28%) and Cm (26%); low resistance levels to Gm (4%), Ntr (3%) and Cip (2%); and no resistance to AMC, Ctx and Caz. Among the individual *E. coli* groups, DAEC and EAEC exhibited higher resistance to Amp, Sxt, Tc and Nal, than EPEC and ETEC (p < 0.05 for each antibiotic). The frequency of resistance of control samples isolated from children without diarrhoea was lower than diarrhoeal samples for Amp (70%, p < 0.05) and Sxt (61%, p < 0.05).
Conclusions: There was a high frequency of antimicrobial resistance of diarrheogenic *E. coli* in Peruvian infants. Resistance patterns were different among the individual *E. coli* groups. Antimicrobial resistance in diarrhoeal samples was more frequent than control samples without diarrhoea, suggesting a possible association of resistance genes with plasmid-associated virulence genes, such as adherence factors.

P2105 Do blood cultures from *E. coli* overestimate the rate of antimicrobial resistance?

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Objectives: Many surveillance systems in Germany, Europe, and worldwide measure the resistance rate in bacteria in hospitals. The systems differ in the inclusion of single departments, antimicrobials, and sources of material. The network GENARS (German network for antimicrobial resistance surveillance) is the only German surveillance system with standardised methods for antimicrobial testing and the inclusion of all sources of material in contrast to the European Antimicrobial Resistance and Surveillance System collecting blood samples only. The GENARS database was investigated under the hypothesis that in comparison to urine the measurement of blood isolates overestimates the resistance rate.
Methods: GENARS consists of 7 German university hospitals, having isolated 20,000 *Escherichia coli* in the period from 2002 to 2007. The isolates were derived from different sources of material and investigated with routine diagnostics. The antibiograms were conducted with a standardised panel of antimicrobials. The minimal inhibitory concentration was interpreted by the DIN standard. The association between type of material and the resistance against the 12 selected therapies were tested by logistic regression analysis simultaneously controlling for age and sex of the patients, laboratories, and the year of investigation.
Results: A statistically significant higher risk for resistance was associated for pathogens detected in blood, in comparison to urine,

for ciprofloxacin Odds Ratio (OR) 1.2 (95% CI 1.1–1.3), levofloxacin OR 1.2 (95% CI 1.1–1.3), co-trimoxal OR 1.3 (95% CI 1.2–1.4), piperacillin OR 1.1 (95% CI 1.0–1.2) and piperacillin/ tazobactam OR 1.1 (95% CI 1.0–1.3). A statistically significant lower risk was associated with ceftazidim OR 0.4 (95% CI 0.4–0.5), cefotaxim OR 0.5 (95% CI 0.4–0.6), and tobramycin OR 0.9 (95% CI 0.8–0.9). No differences were seen for imipenem, meropenem, ampicillin, and ampicillin/sulbactam.
Conclusion: There are differences in the resistance rate, regarding the material tested. This observation may reflect the result of selection by specific antibiotic use. In hospitals urine was presumably investigated from patients who had been treated for urinary tract infection before. Antimicrobial Resistance Surveillance systems based on blood samples may over- or underestimate the resistance rate for single substance groups. Resistance rates are dependent on the sources of the material used.

P2106 Trends in antimicrobial susceptibility of *Escherichia coli* isolates from urology services of 14 hospitals in the Netherlands (1998–2005)

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Objectives: Antibiotic resistance among potential pathogenic micro-organisms is a worldwide problem. To identify the development of resistance actual resistance data of common pathogens are essential to develop therapeutic guidelines. Urinary tract infections are a very common disease both in and outside the hospital, with *E. coli* the most prevalent uropathogen. Optimal antibiotic treatment of UTI may contribute substantially to the control of antibiotic use and antibiotic resistance. We studied trends in antibiotic resistance of uropathogenic *E. coli* during an eight-year surveillance. Ultimately, these data will be used to optimize UTI guidelines.
Methods: During the years 1998–2005, 3360 unique consecutive *E. coli* from urine samples of patients attending the Urology Services were collected in hospitals spread over The Netherlands: 4 in the North-East (NE, n = 1084), 5 in the West (W, n = 1064) and 5 in the South (S, n = 1212).
The antibiotic susceptibility was determined using microbroth dilution according to CLSI-guidelines. The agents tested were those relevant for the treatment of UTI. *E. coli* ATCC35218 and ATCC25922 were used as references.
Results: Amoxicillin resistance remained stable over time (37%-47%) but was higher in the S (44%) as compared to the other regions (40%; $p < 0.02$). Piperacillin resistance increased from 4% to 32% ($p < 0.001$) and differed between the 3 regions (NE 10%, W 12%, S 14%; $p < 0.05$). Fluoroquinolones resistance increased from 6% to 13% over time ($p < 0.01$) and was different between the 3 regions (NE 7%, W 13%, S 8%; $p = 0.000$). Resistance to trimethoprim±sulfamethoxazole remained stable over time (27–37%) although trimethoprim resistance was higher in the S (36%) compared to the NE (32%) and the W (29%; $p < 0.01$). Nitrofurantoin resistance remained low throughout the study (4%-9%) without significant regional differences.
In total 330 strains were resistant for ⩾3 groups of antibiotics (MDR). The percentage MDR was different for the 3 regions (NE 8%, S 10%, W 13%; $p < 0.05$).
Conclusion: Except for piperacillin and the fluoroquinolones, antibiotic resistance over time did not increase, although regional differences were observed. Thus, longitudinal surveillance of antibiotic resistance on an national and regional level is important to keep therapeutic guidelines up-to-date and in line with the resistance of the micro-organisms to be treated.

P2107 Ecology of antimicrobial resistance and virulence in *Escherichia coli* in a rural watershed

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Objective: To examine the phenotypic and genotypic diversity among *Escherichia coli* in a rural watershed in southern Chile. The overall goal of this project is to relate *E. coli* antimicrobial resistance and virulence profiles among dairy farms, wastewater treatment plants, and surface waters in a rural watershed.
Methods: Samples were collected over three years in the Valdivia province of Chile. In Year 1, isolates were collected from cattle (n = 847), wastewater treatment plants (n = 27), and rivers (n = 222). Comparisons of microbial load, antibiotic resistance, and virulence were made among all samples. In Years 2 and 3, samples were collected from surface water samples in 11 of the 12 communities of the province (n = 575 and n = 692, respectively). Distributions of antimicrobial resistance and virulence profiles were compared among sites. In Year 2, all *E. coli* isolates were typed with Box-PCR, and genetic diversity was assessed among sites.
Results: In Year 1, the microbial load in the surface water samples was highest near the wastewater treatment plants. *E. coli* isolates from the river water samples had different antimicrobial resistance profiles than the cattle isolates. Shiga toxin virulence genes were detected only in the cattle isolates. In Years 2 and 3, isolates immediately downstream from the wastewater treatment plants had more resistance than those immediately before the plants. No shiga-toxin virulence genes were detected. Genetic diversity was most variable among communities sampled, but there was considerable variation among sites within communities as well.
Conclusions: *E. coli* isolates from surface waters in this study area had a diversity of antimicrobial resistances. In general, the surface water isolates had resistance profiles that were more similar to the wastewater treatment plant isolates than to the cattle isolates. Virulence genes were only detected in the cattle isolates. These data raise questions about the relative importance of different potential contaminating sources to the surface waters in this area. In addition, the high genetic diversity that was observed among communities suggests that the sources of the *E. coli* might be very local and not spread broadly among the surface waters of the province. Overall, these data will assist in the development of public health interventions in rural communities.

P2108 High proportion of antibiotic resistance in bloodstream infection by nursing-home-acquired *E. coli*

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Background: To evaluate the prevalence of bloodstream infection (BSI) by *E. coli* and the relationship between resistance and source of acquisition.
Methods: We evaluated all the episodes of BSI by *E. coli* in adult patients attended in our hospital during the period 2003–2006. Place of acquisition of bloodstream infection (comunity-acquired [CA], nursing-home-acquired [NH], nosocomial and procedure-related infection), clinical source, antibiotic resistance and temporal trend were evaluated.
Results: A total of 752 episodes were detected; mean age of patients was 71, 8 (SD 17.7) years and 52.8% were women. Number of episodes remained unchanged from 2003 to 2006. Urinary tract was the most common source of bacteraemia (58.5%) followed by biliary tract (15%). According to the place of acquisition of BSI, CA was the most frequent (57.8%) followed by NH (14.2%) and nosocomial (13%). The prevalence differed along the period of the study, decreased in CA (from 66.3% to 56.8%, $p = 0.033$) and increased in NH (from 8.4% to 17.5%, $p = 0.006$). Antibiotic resistance showed an unequal distribution, although the higher rates were observed in quinolone and amoxiciline-clavulanate in NH acquired BSI (Table)

By logistic regresion, ceftriaxone resistance was highly associated to place of BSI acquisition (OR 7.7, IC95 4.4–13.3) and also to age older than 75 (OR 1.7, IC95 1–2.7), and to be male (OR 1.7, IC95 1–2.7).
Conclusions: There is an important increase in *E. coli* BSI NH acquired. Antibiotic resistance is very high, specially in BSI with NH acquisition. Empiric treatment in patient from NH might include the use of carbapenem.

	Resistance, %				
	Community-acquired	Procedure-related	Nosocomial	Nursing-home	Total; p
Ceftriaxone	3.9	25.4	25.0	24.3	12.8%; <0.001
Ciprofloxacin	18.4	46.4	40.0	62.6	31.6%; <0.001
Gentamicin	6.7	19.1	15.0	17.8	11.2%; 0.001
Amoxicillin–clavulanate	12.2	27.3	30	44	21.3%; <0.001
Amoxicillin	57.8	72.7	79.0	74.0	65.2%; 0.002

All isolates were susceptible to ertapenem and imipenem.

P2109 Molecular characterisation of antimicrobial resistant bacteraemic *Escherichia coli* isolates from an Irish hospital

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Escherichia coli is the leading cause of bacteraemia worldwide. Currently, the specific characteristics of *E. coli* associated with bacteraemia are incompletely defined, with limited data linking antimicrobial resistance and virulence.
Objectives: This study aimed to characterise clinical *E. coli* bacteraemic isolates from St. James's Hospital (2004–2005), with regards to antimicrobial resistance genes, phylogenetic background and virulence factors.
Methods: The susceptibility of 221 clinical *E. coli* bacteraemia isolates, to a panel of fourteen antibiotics, was determined using agar microdilutions following CLSI guidelines. Phylogenetic grouping and virulence factor content of each isolate was established using triplex and multiplex PCRs, respectively. The presence of genes encoding TEM, SHV, CTX-M, OXA and plasmidic ampC β-lactamases, as well as the presence of mutations in gyrA, parC and the regulatory region of the ampC chromosomal gene were studied by PCR and sequencing in resistant isolates.
Results: High levels of resistance to aminopenicillins, third-generation cephalosporins, fluoroquinolones and aminoglycosides were detected compared to the European Antimicrobial Resistance Surveillance System data. Beta-lactamases identified included TEM-1 (130), inhibitor resistant TEM's (13), SHV-1 (3) and plasmidic ampC cephalosporinases (4 ACC's and 1 CMY-2). One extended spectrum β-lactamase, CTX-M-15, was also identified. Mutations were present in gyrA (60), parC (55) and ampC chromosomal genes (13). Isolates were predominantly classed as phylogenetic group B2 strains. The majority of resistant and multidrug-resistant *E. coli* belonged to phylogenetic group B2. Resistant isolates, in general, possessed more virulence factors than their susceptible counterparts. The most prevalent virulence factor determinant in the collection was fimH (99%), followed by fyuA (90%) and traT (72%).
Conclusion: This study highlights a positive association between virulence genes and antimicrobial resistance within *E. coli* bacteraemia isolates from St. James's Hospital, contrasting to previous studies. The development of resistant pathogens with increased virulence adds further to already limited treatment options.

P2110 Prevalence of the tetA gene and class I and class II integrons in environmental isolates of *E. coli*

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Objectives: The role the environment plays in the dissemination, maintenance and amplification of antimicrobial resistance is an area of increased study. This study examines the prevalence of tetA as well as class I and class II integrons in *E. coli* isolated from various water bodies in Ireland, including rivers, lakes, effluents and drinking waters.
Methods: Between April 2006 and June 2007, 189 samples from various sources (hospital effluent (HE), city effluent, source and piped drinking water, sea water, outflow from a secondary wastewater treatment plant, rivers and lakes) were collected. All samples were screened for the presence of antimicrobial resistant *E. coli* by quantitative culture in the presence and absence of cefotaxime (CTX), ciprofloxacin (CIP), cefoxitin (FOX), ampicillin (A), streptomycin (S), tetracycline (T) and sulphonamides (Su) as appropriate. Antimicrobial resistance was confirmed by identification and antimicrobial susceptibility testing (CLSI disk diffusion) of representative isolates. Isolates resistant to 4 or more antimicrobials were screened for the presence of tetA and class I and class II integrons by PCR using specific primers and protocols as previously described.
Results: Fifty-six *E. coli* isolates were isolated from; HE (23), city effluent (4), treated effluent from a waste water treatment facility (6), sea water (3), source water (11), piped drinking water (6), rivers (1) and lakes (2). Thirty isolates (54%) harboured the tetA gene, twenty-nine isolates (52%) harboured a class I integron, and 3 isolates (5%) (3) harboured a class II integron. The prevalence of tetA, class I and class II integrons and at the various sites tested was as follows; HE 52% (12), 61% (14), 4% (1), City effluent 75% (3), 75% (3), 0% (0), lakes 50% (1), 100% (2), 0% (0), piped water 83% (5), 50% (3), 17% (1), rivers 100% (1), 0% (0), 0% (0), sea water 66% (2), 100% (3), 0% (0), source water 45% (5), 27% (3), 0% (0), treated effluent 17% (1), 17% (1), 17% (1). Of the isolates harbouring tetA (30), 17 possessed a class I integron and 1 possessed a class II integron. One isolate collected from HE harboured both class I and class II integrons.
Conclusion: Class I integrons are widely disseminated in environmental isolates of *E. coli*, with a stronger correlation of tetA with class I integrons than class II integrons.

Epidemiology of vancomycin-resistant enterococci

P2111 Plasmid characterisation of vancomycin-resistant *Enterococcus faecium* strains from different continents (1986–2007)

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Objectives: Vancomycin-resistance (VR) among *E. faecium* (Efm) isolates is frequently transferable by conjugation although little is known about the molecular epidemiology of transferable plasmids in this species. We have analysed VREfm plasmids recovered in the last two decades in different continents.
Methods: Ninety-six VR (88 VanA, 8 VanB) *E. faecium* isolates were collected from 14 countries (Portugal, Spain, France, Greece, Germany, the Netherlands, UK, Hungary, Norway, Denmark, Serbia, USA, Brasil and Australia) over a 21-year period (1986–2007). They mostly belong to CC17 and are representative of VREfm clinical outbreaks and community isolates in these areas. Plasmid characterisation included determination of size and content, comparison of ClaI-RFLP patterns, identification of relaxases by using a Multiplex-PCR-based relaxase (rel) typing method (Goicoechea et al, ECCMID submitted), rep initiator proteins (rep) and toxin-antitoxin systems (TA) by PCR. Results were confirmed by hybridisation and sequencing.
Results: A high diversity of plasmid content was observed (1–6 /cell, 2–350 kb). Conjugation was successful in 80% of strains, sometimes involving more than one VR plasmid (25–250 kb). VREfm isolates from different continents harboured plasmids containing relaxases from pEF1 (92%), pCIZ2 (48%) or pHTbeta (25%); rep proteins from pRUM (81%), pRE25 (60%), pHTbeta (50%), pCIZ2 (48%)and Inc18 (41%); and Axe-Txe (31%) or omega-epsilon-zeta systems (14%). Relaxases from pRUM, pRE25, and pAD1 were rarely detected. Differences among

VREfm plasmids from different geographical locations were observed (rel and rep types content and presence of Axe-Txe system).
Conclusions: VREFfm isolates recovered in the last two decades contained plasmids with similar relaxases and rep initiator proteins suggesting a common evolutionary trend in CC17. Conjugative plasmids containing relaxases from pEF1 seem to have influenced the spread of vancomycin resistance worldwide in last decades.

P2112 Plasmid characterisation of vancomycin-resistant *Enterococcus faecalis* strains from different continents (1989–2004)

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Objectives: Vancomycin resistant (VR) enterococci are more associated with *E. faecium* than *E. faecalis* (Ef). Plasmid diversity among VREf was analysed in order to better understand the plasmid ecology and the scarce dissemination of vancomycin resistance in this species.
Methods: We analysed 44 VREf-vanA isolates (19 PFGE types, 13 ST types containing 7 Tn1546 types) from 8 countries (Portugal, Spain, Italy, UK, Cyprus, Serbia, USA and Argentina), recovered from 1989 to 2004. They are representative isolates causing well characterised outbreaks, most of them belonging to HiRCC (CC2, CC9 and CC87). Plasmid characterisation included: (i) size and content (S1-hybridisation), (ii) EcoRI/ClaI-RFLP comparison, (iii) identification of relaxases (rel) by a multiplex-PCR typing method (Goicoechea et al, ECCMID submitted), (iv) identification of rep initiator proteins (rep), (v) detection of 5 toxin-antitoxin systems (TA) by PCR. Results were confirmed by hybridisation and sequencing.
Results: A variable number of plasmids (1–4/cell) from 25 to 300 kb was observed, some isolates containing more than one vanA-plasmid. RFLP plasmid patterns were highly diverse with some common types identified in different countries over years.
VREf isolates contained a diversity of rel [rel pAD1 (93%), rel pRE25 (32%), rel pRUM (32%), rel pCF10 (11%), rel pAMalpha1 (9%), rel pHTbeta (9%), rel pEF1 (5%)], rep [repAD1 (80%), rep Inc18 (61%), rep pRE25 (61%), rep pAM373 (18%), rep pHTbeta (9%)] and TA systems [par (68%), and Axe-Txe (2%)]. Most vanA plasmids hybridised with probes for rel pAD1, rep pAD1, rep pRE25 and par genes, although plasmids containing rel pHTbeta (variable rep and TA content) were also detected in some countries. Differences in plasmid size and rep/rel/TA content between wild type and corresponding transconjugants were frequently observed.
Conclusions: VREf plasmids are mainly associated with derivatives of the narrow host pheromone-responsive pAD1 from *E. faecalis*. Diversity in the rep, rel and TA content in the same plasmid platform highlights the modular structure of enterococcal plasmids and the impact of genetic exchange in the adaptation and evolution of *E. faecalis*.

P2113 Evaluation of virulence and resistance genes structure in vancomycin-resistant *E. faecium*

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Objectives: To analyse the presence of virulence genes and Tn1546 structure of *E. faecium* isolates resistant to vancomycin presented by different PFGE types, collected from patients with haematological malignancies.
Methods: Clonal relatedness of *E. faecium* strains, harbouring VanA genes, (October 2004 to May 2007) was evaluated by PFGE. First strain of each PFGE type was analysed. Presence of virulence traits, such as Enterococcal surface protein (esp), gelatinase (gelE), cytolysin (cylA), hyaluronidase (hyl), aggregation substance (agg), and the backbone structure of Tn1546 were studied using PCR technique.
Results: Typing of 138 *E. faecium* strains revealed 24 PFGE types. Six types contained more than one strain and formed epidemic clones. Two of them predominated, 85 (62%) and 12 (9%) isolates belonged to types A and F. Other 18 strains were sporadic, each of them presenting one PFGE-type. PCR analysis of Tn1546 showed that only 16% (8/50) of epidemic isolates had deletions in either ORF1, VanX or VanY genes. Other epidemic strains harboured the prototype Tn1546. Higher diversity of Tn1546 structures was found in sporadic isolates, 7/18 (39% vs 16%, p = 0.048), which had large deletions in ORF1 genes and insertions between VanS and VanH. There were six different types of Tn1546 found. Various presence of virulence traits was detected in sporadic and epidemic isolates. No asa or cylA were present. GelE gene was found only in 2% (2/50) of epidemic and in 6% (1/18) of sporadic strains. Esp and hyl genes were detected in 28% (14/50) and 38% (19/50) of epidemic, in 33% (6/18) and 28% (5/18) of sporadic isolates. Among the epidemic clones the prevalence of esp-positive isolates was detected for clone A 43% (13/30), compared to 5% (1/20) for other epidemic clones (p = 0.005).
Conclusion: The spread of vancomycin-resistant *E. faecium* was polyclonal with two predominant clones. Transmission of transposons among epidemic and sporadic strains could also occur. Prevalence of prototype Tn1546 elements in epidemic strains observed, could explain the higher ability, compared to sporadic isolates, to transmit resistance genes among different epidemic strains and survive in the hospital environment. Distribution of virulence factors was estimated to be diverse, with a higher frequency of esp gene in dominating epidemic clone A.

P2114 Prevalence and epidemiology of vancomycin-resistant enterococci in a Russian haematology centre

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Objectives: To determine the epidemiology of clinical strains of vancomycin-resistant *Enterococcus faecium* (VRFE) isolated from patients with haematological malignancies. To explore the prevalence of VREF strains in hospitalised patient of Haematology Unit (HU), Intensive Care Unit (ICU), and to determine the clonal relationship.
Patients and Methods: Clinical isolates of VRFE were collected from January 2004 and November 2007. Patients, who stayed in HCU and HU for seven days and more, than 28 days were investigated. New patients admitted to the HU were screened. Identification of isolates and presence of vancomycin resistance genes was established using PCR. Macrorestriction analysis of SmaI digests was performed by pulsed-field gel electrophoresis. PFGE patterns were then compared using BioNumerics 3.0.
Results: A total of 160 VREF clinical strains were isolated, 13 (8%) in 2004, 49 (31%) in 2005, 62 (39%) in 2006, and 36 (22%) in 2007. VREF isolates carried vanA (96%) or vanB genes (4%) and were multiresistant to antimicrobial agents, except linesolide (MIC from 1 to 2 mg/L). Strains of VREF were prevalent in two departments – ICU (34%) and HU (32%). Bloodstream infections caused by VREF were observed in five (3.1%) patients, one (2.0%) in 2005 (ICU), one (1.6%) in 2006 (HU) and three (8.3%) in 2007 (ICU, HU, and other unit). In the ICU the carriage of VREF increased from 22% (10/46) in patients staying for seven days to 82% (9/11) in patients staying for more than 28 days. In the HU the detection of VREF increased from 7.2% (7/96) in patients staying for seven days to 26% (16/61) in patients staying for more than 28-day. PFGE type A strains prevailed in patients in ICU (75%) and HU (83%). Since July 2006 to June 2007 none of the patients newly admitted to the HU (n = 70) carried VREF.
Conclusion: VREF strains were higly of distributed among patients hospitalised in two departments (HU and ICU). One predominant clone was disseminated in HU and ICU, suggesting intrahospital transmission. Detected predominant clone in blood culture isolates may be a threat of possible transition of VREF colonisation to invasive disease.

P2115 **vanB2-Tn5382 typing in enterococcal clones disseminated around 16 hospitals in Chile**

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Objectives: To analyse the composition of the genetic element Tn5382 associated with the vanB2 genotype in selected enterococcal clones disseminated around 16 different Hospitals in Chile, as well as the presence of other resistance, virulence and bacteriocin genes.
Methods: Seven unrelated PFGE-SmaI clones (2 *E. faecalis* and 5 *E. faecium*) corresponding to 10 *E. faecalis* and 60 *E. faecium* vanB2 isolates were studied. Strains were collected at the National Reference Institute in Santiago (ISPCH) from 16 different hospitals of four regions of Chile. Antimicrobial susceptibility was determined by the agar dilution method. Presence of ISEnfa110 or ISEnfa200, as well as the vanX-ORFC fragment or the antimicrobial resistance genes erm(B), tet(M), tet(L), aph(3′)-III and aac(6′)-aph(2″) were tested by PCR. Similarly, esp, hyl, gelE, fsrA, fsrB, fsrC, cylLLS, cylA, cylB, cylM, and agg virulent factors were tested in all vanB2-enterococcal clones by PCR. Amino acid changes in PBP5 protein was studied in one penicillin-resistant *E. faecium* clone by PCR and sequencing. The purK allele was analysed by PCR and sequencing. Bacteriocin production was analysed using 8 indicator bacteria and bacteriocin structural genes were checked by PCR.
Results: All five vanB2 *E. faecium* clones harboured the Tn5382 element with positive amplifications for ISEnfa110 upstream of the vanRB gene, whereas ISEnfa200 was not detected between the intergenic region vanSB-vanYB. Negative results were obtained for both ISEnfa100/200 elements in the two vanB2-*E. faecalis* clones. The sequence of vanX-ORFC fragment corresponded to the described sequence 203412. Eight amino acid changes were detected in PBP5 protein in one ampicillin-resistant *E. faecium* clones respect to the reference one. Almost all enterococcal clones tested harboured the resistant genes erm(B), tet(M), aph(3′), and aac(6′)-aph(2″), and the *E. faecalis* ones also contained all the virulence tested factors. The purK1 allele was found in all *E. faecium* clones. Bacteriocin production was detected in two *E. faecium* and two *E. faecalis* clones, showing activity against *L. monocytogenes*, among others, and they harboured the entA gene.
Conclusions: different vanB2 clones of *E. faecium* and *E. faecalis* have been disseminated in hospitals of four different regions of Chile and most of them harbour a wide variety of antibiotic resistance genes and virulence genes.

P2116 **Alert patogeny among patients in a medical university hospital, Gdansk**

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Objectives: The aim of this study was to analyse the occurrence of alert pathogens recovered from patients who were hospitalised in the Hospital of Medical University Gdansk
Methods: We analysed 230678 microbiologic records from 2002 to 2006.
We looked for alert pathogens like ESBL + (Extended-Spectrum Beta-Lactamases), MRSA (Meticillin-resistant *Staphylococcus aureus*), VRE (Vancomycin-Resistant *Enterococcus*). Strains were identified by classical method and VITEK and VITEK 2 cards (BioMerieux). Production of ESBL was detected by double disk method. Only first alert pathogens from diagnostic and not epidemiological specimens were analysed.
Results: ESBL+ isolates were recovered from 1317 patients.(7.02 100 patient/days) VRE isolates were recovered from 356 patients, (1.98 100 patient/days) MRSA isolates were recovered from 129 patients (0.46 100 patient/days).
ESBL+ were recovered in 2002 from 350 patients (0.068 100 patient/days), 2003 – 514 (0.077), 2004 – 306 (0.06), 2005 – 344 (0.071), 2006 – 471 (0.086). VRE were recovered in 2002 from 76 patients (0.019 100 patient/days), 2003 – 68 (0.017), 2004 – 74 (0.02), 2005 – 61 (0.018), 2006 – 77 (0.024). MRSA were recovered in 2002 from 40 patients (0.01 100 patient/days), 2003 – 24 (0.006), 2004 – 16 (0.004), 2005 – 26 (0.008), 2006 – 23 (0.007).
Among ESBL+ isolates *K. pneumoniae* was the most often isolated 35%, *E. coli* 28%, *E. aerogenes* 11%, *K. oxytoca* 7%, *E. cloacae* 6%, *C. freundii* 3% and *S. marcescens* 3%.
Isolates of ESBL strains were recovered from urine (31%), respiratory tract (15%), wound specimens (10%), and blood (3%).
Among VRE isolates the most often were *Enterococcus faecium* 95%, *E. faecalis* 1% and *E. gallinarum* 1%.
They were recovered from respiratory tract (34%), urine (26%), blood (21%) and wound specimens (16%).
MRSA isolates were recovered from respiratory tract (54%), wound swabs (36%) and blood (4%).
Conclusions: High percentage of ESBL carriage complicates infection control and treatment of common urinary tract infection. ESBL-producing strains are the most often isolated and cause the greatest concern. There are not significant differences in the rate of their incidence throughout the studied period. VRE are quite seldom isolated from diagnostic specimens however their carriage rate which was not analysed here is about three times higher. MRSA infected patients tend to come from other hospitals and we have not discovered any cross infections recently due to successful infection control programme.

P2117 **Molecular typing of the resistance plasmids of a vancomycin-resistant enterococci nosocomial outbreak in Spain**

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Objectives: Vancomycin-resistant enterococcal outbreaks are unusual in Spain. However, 31 clinical isolates of VanA *E. faecium* showing high-level resistance to vancomycin, ampicillin, erythromycin, ciprofloxacin and aminoglycosides were reported in our hospital between October 2002 and April 2004. PFGE typing indicated the presence of three different epidemic clones, A, B and C (25, 5 and 1 isolates, respectively). The aim of this work was to investigate the diversity of the plasmids carrying antibiotic resistance in our VRE isolates.
Methods: Plasmids were characterised using a new Multiplex-PCR relaxase typing method described in an accompanying communication. The results were compared to the ones obtained by PCR using oligonucleotides targeting the replication initiator sequences (rep) of known enterococcal plasmids. Sequencing of the positive amplicons and S1-PFGE and hybridisation analyses were done for confirmation of the results.
Results: Three to seven different plasmids were identified in our VRE isolates, independently of the epidemic clone considered. Antibiotic resistance genes vanA and ermB were generally associated to the same plasmid. All isolates were shown to harbour pEF1- and pCIZ2-related plasmids, according to the relaxase encoded and pCIZ2- and pRUM-like plasmids, based on the rep sequence. Clon A isolates were shown to specifically harbour pMG1 related plasmids based on both, relaxase and rep sequences, and pRE25-like plasmids (only rep sequence). Presence of pEFNP1-related plasmids was exclusive of clon C.
Conclusions: pEF1-related relaxases and pRUM-like rep sequences are associated to plasmids carried by all vancomycin resistance *E. faecium* isolates in our institution, suggestive of a dissemination among the different clones. PCR-based typing methods will facilitate studies about prevalence of particular resistance plasmids in different environments.

P2118 **Surveillance of glycopeptide-resistant enterococci in faecal samples from hospitalised patients during two years in a tertiary hospital**

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Objectives: The surveillance of glycopeptide resistant Enterococci (GRE) strains isolated from faecal samples from hospitalised patients in

General Hospital of Athens "G. Gennimatas" during a two year period (27/11/05–31/10/07).

Methods: In a prospective study 3253 faecal samples were examined for GRE. The origin of the samples was: Haematology, Neurosurgery, Nephrology, Plastic-Burn Unit (PBU), Intensive Care Unit (ICU) and Internal Medicine Department, as well as faecal samples from all wards which were sent to the laboratory for *Clostridium difficile* (Cd). GRE strains were isolated on esculin azide agar and Cd strains on Cycloserine Cefoxitin blood agar (BD). The strains were identified by VITEK 2 automated system (bioMerieux).

Results: During the study period 561 GRE strains were isolated from faecal samples (561/3253, 17.24%). The strains were identified mainly as *E. faecium* (553/561, 98.6%) and only 8 strains were *E. faecalis* (8/561, 1.4%). The distribution of the strains per clinical department was as follows: Internal Medicine 209 (37.2%), Haematology ward 172 (30.7%), PBU 63 (11.2%), Nephrology Ward 54 (9.6%), ICU 43 (7.7%) and Surgical Department 20 (3.6%). Bacteraemia by GRE strains was observed in thirteen patients who carried the same strains in their stools. All cases of bacteraemia were due to *E. faecium* and were recovered from PBU (6), Internal Medicine Department (4), Haematology Ward (2) and ICU (1). Co-existence of GRE and Cd was present in 54 cases (54/561, 9.6%) and in one case the co-existence was followed by bacteraemia.

Conclusions:

1. The enteric carriage of GRE was approximately 17% of our patients
2. Most of the GRE strains were identified as *E. faecium*
3. In 13 cases, six of which in PBU patients, carriage of GRE led to bacteraemia
4. Co-existence of GRE and Cd suggests that specimens submitted for Cd must also be screened for GRE
5. Antibiotic policy and strict enteric precautions should be implemented to restrict the GRE carriage

P2119 Clonality of *Enterococcus faecalis* isolates from infective endocarditis patients at a Danish hospital

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Objectives: To investigate the clonality of *Enterococcus faecalis* isolates from infective endocarditis (IE) patients at Aalborg Hospital in Denmark. The issues addressed include the apparent contradiction between the ability to cause IE and the presumed poor selective advantages as such infections often lead to rapid host death and poor transmission to new hosts.

Methods: For this study, we used 27 *E. faecalis* isolates from patients with definite IE according to the modified Duke criteria. These isolates were collected prospectively over a 7-year period from 1996 to 2002 at Aalborg Hospital in Denmark. The clonality of the isolates was inferred by using multilocus sequence typing (MLST).

Results: The MLST data revealed 15 sequence types (STs) among the 27 isolates. Fourteen isolates clustered in 6 groups of single-locus variants (SLVs), of which 10 were members of clonal complexes (CC40, CC21, and CC25), whereas 13 isolates clustered in 7 singleton STs. CC25 (8 isolates) appears to be a high-risk enterococcal clonal complex associated with human bloodstream infections in Europe, whereas CC40 (1 isolate) and CC21 (1 isolate) exhibit larger ecologic diversity according to their host and geographic origin, and commensalism/virulence traits.

Conclusion: The results demonstrated surprising diversity among *E. faecalis* isolates causing IE, despite their narrow time-span and geographic range. The virulence trait was randomly distributed within *E. faecalis*, supporting the idea that virulence might be coincidental to a commensal lifestyle. However, it is also possible that horizontal gene transfer of virulence-related factors has lead to the emergence of clonal complexes (i.e., CC25) associated with an increased ability to cause IE. This work will allow further epidemiological, genetic, and phenotypic studies devoted to test how virulence evolves in *E. faecalis*.

P2120 How to analyse and interpret infectious disease data: statistical insights into the epidemic nature

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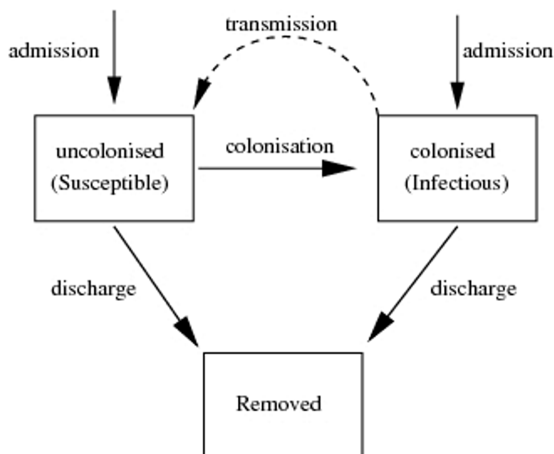
Objectives: Highly infectious diseases are often analysed in the same way as non-communicable diseases, ignoring basic epidemiological assumptions, as e.g. that the acquisition depends on contact transmission. The objective is to present adequate statistical methods based on individual patient data which take this epidemic nature into account. The main focus is the time-varying transmission rate: the rate with which an infectious individual has close contacts with other patients in the hospital unit. Discharge has to be taken into account as a competing event.

Methods: The statistical time-to-event analysis is based on an appropriate compartmental model which describes the transmission dynamics of the epidemic process. The well-known S-I-R model (Susceptible-Infectious-Removed) with migration (admission and discharge) is applied to the hospital setting (figure). The Reed-Frost assumption specifies that each colonised individual creates infectious contacts independently. For the statistical modelling, generalised linear and additive Aalen models are used.

Results: These methods are applied to simulations and individual patient data of an outbreak of the pathogen Vancomycin-Resistant-Enterecocci (VRE) in an onco-haematological unit at the University Medical Centre Freiburg in Germany. All patients were followed up for 105 days and 41 patients acquired a VRE during their hospital-stay. Graphics show the contact pattern with possible cross-infections, the time-varying transmission and discharge rates. The effect of isolation on the transmission rate is quantified.



Conclusion: With our methods, an epidemic spread on the unit-level in a hospital can be described in an appropriate way and it will give valuable informations for clinicians working in infection control and public health.

P2121 Epidemiology of vancomycin-resistant *Enterococcus faecium* in Greek hospitals by PFGE and MLST

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Objectives: *Enterococcus* spp. is an important cause of nosocomial infections worldwide. Nowadays, the dissemination of vancomycin-resistant enterococci (VRE), remains a major problem in hospital environments. The aim of this study was to investigate the epidemiological relatedness of vancomycin-resistant *Enterococcus faecium* isolated from clinically significant specimens in different Greek hospitals.

Materials and Methods: A total of 100 vancomycin-resistant *Enterococcus faecium* (VRE), isolated from hospitalised patients

throughout Greece were included. These isolates were recovered from urine, stool, blood and pus. Identification to the species level was done by PCR based on sodA gene, while vancomycin-resistance was associated with vanA gene. The clonality of isolates was tested by PFGE, after digestion of DNA with SmaI. To increase the sensitivity of our approach, due to the difficulty of determining small differences in band patterns by PFGE, strains of similar cloning patterns were also subjected to Multi Locus Sequence Typing (MLST) and the results compared. Detection of pathogenic genes esp, gel, as, ace and efa genes was performed by PCR, using specific primers.
Results: From the 100 VRE isolates, twenty-two different PFGE clones were noticed. Throughout each hospital, similar clones were observed. Between strains with similar PFGE pattern, differences were observed after MLST typing. The majority of isolates carried the esp gene, and they were negative for all the remaining genes.
Conclusions: PFGE remains a technique that is usefully applied for the primary surveillance of epidemic clones in hospitals, while MLST is an extremely important tool and should be used in conjunction with PFGE.

P2122 Molecular analysis of vancomycin-resistant enterococci isolated in Debrecen, Hungary

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Objectives: Although vancomycin resistant enterococci (VRE) are common nosocomial pathogens, they have been rarely isolated in Hungary. The aim of the present study was to explore the prevalence of VRE colonising hospitalised patients in the hospitals of the Medical School at the University of Debrecen, and to identify the van resistance genes.
Methods: 3946 clinical samples collected between January 2004 and November 2006 were found to contain various enterococcus strains by the Bacteriological Diagnostic Laboratory. These isolates were screened on 6 mg/l vancomycin containing BHI agar plate according to CLSI (NCCLS). 44 screen-positive isolates were tested by E-test for vancomycin and teicoplanin resistance. Suspicious colonies were also investigated by the VITEK 2® system. PCR amplification of the *Enterococcus faecalis* and *Enterococcus faecium* ddl genes as well as *Enterococcus casseliflavus* and *E. faecalis* sodA genes were used for species identification. Multiplex PCR was preformed for the detection of the vanA, vanB, vanC1/C2, vanD, vanE and vanG genes. VanC1 and vanC2 were differentiated by a method based on the restriction digestion of the PCR products by HindIII and SalI enzymes. The van gene carrying PCR products were confirmed by DNA sequencing.
Results: We identified the vanC1 resistance gene in 4 clinical samples: 1 *E. faecalis* and 1 *E. casseliflavus* from urine, and 1 *E. faecalis* from wound secretion. In one urine sample both *E. faecalis* vanC1 and *E. casseliflavus* vanC2 resistance gene carrying bacteria were found. A highly resistant sample (vancomycin MIC > 256) from femoral wound was found to contain vanA carrying *E. faecalis*.
Conclusion: This is the first report on the occurrence of the vanC1 resistance gene in Hungary. Furthermore, we found a new vanC2 variant, having a sequence that is slightly different from the one reported in the database. We conclude, that molecular screening of the resistance genes is important in the diagnostics of VRE, and the identification of the carrier by PCR is more reliable than standard biochemical methods

P2123 Prevalence of vancomycin-resistant enterococci colonisation in gastro-intestinal tract of hospitalised patients in a hospital, Esfahan Province, Iran

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Introduction: Vancomycin-resistant enterococci (VRE) are the most common nosocomial pathogen worldwide. Colonisation with VRE can lead to serious infection, that some like VRE sepsis can be fatal. Because VRE are dangerous and important pathogens, we are going to determine the prevalence of VRE among patients admitted in infectious, surgery wards and ICU in AL-Zahra Hospital in Isfahan, Iran.
Methods: 100 patients from infectious and surgery (post-operative patients) wards and ICU were selected by simple sampling method. Stool specimens were taken from selected patients and cultured in VRE selective media (bile-esculin agar plate with 6 of vancomycin) and Gram-positive cocci from black colonies were inoculated to the tryptase soya broth+ 6.5% Nacl and again Gram-positive cocci were inoculated to bile esculin and finally MIC (minimal inhibitory concentration) evaluated by E-Test for detection of VRE.
Data were analysed by ANOVA and Chi-square tests using SPSS V13/Win.
Results: 58 out of 100 patients, had positive culture for enterococci. 16 out of 58 were female (27.6%) and 42 (72.4%) were male. Among 58 culture, 17 (29.3%) were highly resistant to vancomycin. There was significant relation between previous antibiotic therapy especially vancomycin and VRE in this study ($P = 0.02$). Most of the patients (74.1%) with negative culture for enterococci had GI surgery. Most of culture positive patients (46%) had been in infectious ward. No significant relation accompanied between VRE and sex, GI surgery and admission ward in this study.
Conclusion: Results of this study suggest that previous antibiotic therapy especially vancomycin and β-lactam is a major risk factor for colonisation with VRE. Prevalence of VRE was high in our study and this problem is very important in epidemiology of hospital infections. Considering the fact that there is no substitute agent for vancomycin in our country, it is essential to obtain necessary guidelines regarding over treatment with antibiotics specially vancomycin.

P2124 Clonal spread of vancomycin-resistant enterococci in Swedish broiler chickens

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Objectives: In Sweden, the occurrence of acquired resistance among *E. coli* and *Enterococcus* spp. isolated from intestinal content from healthy broiler chickens, pigs and cattle is investigated on a regular basis within the Swedish Veterinary Antimicrobial Resistance Monitoring programme (SVARM, www.sva.se). Within SVARM a total number of 1853 broiler samples have, since 2000, been cultivated both on Slanetz-Bartley agar and on Slanetz-Bartley agar supplemented with 16 mg/l vancomycin. Using non selective media only four VRE have been isolated through the years. With selective media the number of positive samples has however been higher and the proportion of positive samples has gradually increased from 0.6% (2000) to 28.4% (2006). All VRE isolates have been identified as *Enterococcus faecium* with MIC of vancomycin of >64 mg/L and carrying the vanA gene cluster using phenotypic methods, micro-dilution method and PCR. Similarities in antibiogram, phenotypic characteristics and macro restriction patterns indicate clonality. The aim of present study was to confirm that the increase of VRE is due to clonal spread of a particular VRE strain.
Methods: The genetic relatedness of the first two VRE isolates, found in 2000, and a representative set of 46 randomly selected isolates between 2001 and 2007 was determined using multi locus sequence typing (MLST).
Results: All 46 randomly selected isolates revealed sequence type (ST)310, while the two 2000 isolates revealed ST13 and a new ST370. ST310 was previously isolated from a Norwegian poultry sample, whereas ST13 was isolated in 3 Dutch poultry samples.
Conclusions: This study revealed that the increased occurrence of VRE in Swedish broilers since 2000 is due to spread of a single clone (ST310). Apparently the clone persists within and spreads between poultry farms all over Sweden. This is a concern since a reservoir of the vanA gene is generated and maintained in broilers, which could spread to humans via the food chain, and can potentially spread further into hospital settings. Further research is needed to investigate possible means to minimize occurrence of VRE in broilers.

P2125 **Rapid screening protocol for the detection of glycopeptid resistant enterococci using enterococcosel broth, enterococcosel- and chromID™ VRE agar with subsequent molecular GenoType enterococcus test**

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Introduction: Rapid and accurate screening protocols are required to control spread of vancomycin-resistant enterococci (VRE) and for the successful management of colonised or infected patients.
We compared the performance of Enterococcosel (EC) broth with EC agar and subsequent PCR for the rapid detection of VRE in screening specimens (study phase I). In study phase II a new selective agar for VRE-detection (chromID™ VRE, bioMérieux, Nürtingen) was compared to EC broth.
Material and Methods: 309 screening specimens were tested (rectal swabs, stool, urine, wound and others) in study phase I and 80 specimens in study phase II, respectively. The material was inoculated in trypticase soy broth and then transferred to Columbia Blood agar, EC agar with 6 mg/l vancomycin (Becton Dickinson, Heidelberg, Germany), EC broth with 6 mg/l vancomycin and to chromID VRE. Each positive EC broth and positive agar media were investigated with the DNA-based strip assay Genotype Enterococcus (Hain Lifescience, Nehren, Germany) for the simultaneous identification of four *Enterococcus* species and the associated van-genes (vanA, B, C1, C2/3) in 4 hours.
Results: In study phase I 61 VRE isolates were detected with the EC broth, 58 with the EC agar and 29 with Columbia blood agar, respectively. 26 (43%) of the VRE-containing broth were positive on day 1, 35 (57%) on day 2. On EC agar 21 (36%) of the VRE isolates were positive on day 1 and 37 (64%) on day 2. With regard to study phase II EC broth detected 25 VRE isolates, chromID VRE 22 isolates. 11 (44%) of the VRE-containing broth were positive on day 1, 14 (56%) on day 2. On chromID VRE 9 (41%) of the VRE isolates were positive on day 1 and 13 (59%) on day 2. VRE-detection with the molecular GenoType Enterococcus test directly from the different selective media performed excellent. Inhibition only occurred in three cases with EC broth containing stool specimens.
Conclusions: Selective broth and agar media with subsequent PCR test showed excellent results in detecting VRE from screening specimens in at least two days.
chromID VRE proved to be a sensitive, specific and rapid tool for the detection of VRE from screening specimens. In addition to glycopeptide-resistance enterococcal species differentiation is possible with the chromID VRE agar due to different colony colour.
Molecular detection of the van genotypes from EC broth and selective agar media with the Genotype Enterococcus assay showed excellent results.

MRSA and VRE: laboratory detection

P2126 **The efficacy of chromogenic meticillin-resistant *Staphylococcus aureus* agar for inocula preparation in automated susceptibility testing**

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Objectives: In many diagnostic laboratories chromogenic MRSA agar is used to aid rapid detection of MRSA, making reporting of MRSA within 24 hours of specimen submission possible. Further susceptibility testing is usually performed from a subculture onto blood agar (BA) and results reported at 48–96 hours. The use of automated susceptibility testing techniques is increasingly common, with inocula prepared from a blood agar recommended. In order to reduce costs and improve speed of susceptibility testing reporting, we compared susceptibility testing results on PhoenixTM using standard BA, and two branded Chromogenic MRSA agars for preparation of the inocula.
Methods: 30 previously confirmed MRSA. All strains were subcultured onto BA, Oxoid Chromogenic MRSA agar (O) and BioMerieux MRSA ID agar (B). After 24 h the cultures were used to prepare inocula for susceptibility testing of 22 antimicrobials in PhoenixTM (Becton Dickinson). 3 strains were repeated on 3 occasions to predict internal error. The minimum inhibitory concentration (MIC) plus interpretations were compared for each strain and agar used. Minor, major and very major errors were used to denote: change in MIC but not interpretation; change in MIC with interpretation change from sensitive (S) to intermediate (I) or resistant (R) and change in MIC with interpretation change from R or I to S.
Results: When using O agar, 8 out of 22 antimicrobials showed minor, major or very major errors. 10 antimicrobials were affected by the use of B agar (See table). Chloramphenicol (C), trimethoprim (T) and vancomycin (V) MICs varied with repeated testing, with only T exhibiting major errors. When using O agar, major errors were seen in C, levofloxacin (L), mupirocin (M), T and very major errors seen in gentamicin (G) and L. When using B agar major errors were seen in C, L, M and T, whereas very major errors were seen in only G.

	Percentage error rate		
	Minor	Major	Very major
Oxoid MRSA agar			
Amox/Clav	16.6		
Chloramphenicol	2.4	3.3	
Gentamicin			3.3
Levofloxacin	6.6	3.3	6.6
Mupirocin	3.3	3.3	
Mupirocin high	3.3		
Teicoplanin	3.3		
Trimethoprim	21.9	43.3	
Vancomycin	11.5		
BioMerieux MRSA ID agar			
Amox/Clav	10		
Chloramphenicol		3.3	
Clindamycin	3.3		
Fusidic acid	3.3		
Gentamicin			6.6
Levofloxacin	3.3	3.3	
Mupirocin	3.3	3.3	
Teicoplanin	3.3		
Trimethoprim	48.6	1.9	
Vancomycin	31.9		

Conclusions: The direct use of chromogenic MRSA agar for susceptibility testing would improve reporting times of susceptibility testing results for MRSA. No major or very major errors were seen when testing antimicrobials commonly used to treat MRSA infection. Chromogenic MRSA agar can be used directly for antimicrobial susceptibility testing providing interpretation of C, L, G, T and M are taken with care.

P2127 **Rapid detection of meticillin-resistant and meticillin-susceptible *Staphylococcus aureus* by real-time PCR directly from positive blood culture bottles**

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Background: Recently, a new automated system Xpert MRSA/SA assay (Cepheid, USA) has been developed for direct detection of *Staphylococcus aureus* and determination of meticillin resistance in clinical specimens. This real time PCR is a fully-automated molecular testing platform which combines sample preparation, multiplex PCR assay and nucleic acid analysis. The objective of this study was to evaluate the performance of the Xpert MRSA/MSSA assay directly from positive blood culture bottles.

Material: Real time PCR assays were prospectively performed directly on signal positive blood cultures (Bactec 9240, Beckon Dickinson, USA). 50 μl of blood culture broth were transferred into the elution reagent tube. The PCR assay was performed according to the manufacturer's instructions. Phenotypic identification and antibiotic susceptibility testing were performed in parallel. Suspected colonies of *S. aureus* were identified by latex slide agglutination and coagulase testing. Oxacillin susceptibility was tested by cefoxitin disk diffusion according to CLSI recommendations.
Results: A total of 197 signal positive blood cultures from 87 patients were tested by real time PCR. *S. aureus* isolates including 22 MRSA and 32 MSSA which were all correctly detected by Xpert assay. The sensitivity and specificity of PCR compared with phenotypic methods were both 100%. The PCR assay provided results in less than 2 hours versus 48 hours for conventional methods.
Conclusion: The Xpert MRSA/MSSA assay correctly detected 100% of MSSA and MRSA isolates from positive blood cultures in less than 2 hours. Prospective studies are needed to evaluate the clinical impact of this assay for rapid implementation of targeted treatment for *S. aureus* bacteraemia.

P2128 Rapid detection of meticillin-resistant *Staphylococcus aureus* in critical care units

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Objectives: The Baclite flex is a rapid culture-based MRSA detection system with a laboratory processing time of 5 hours. We evaluated the effect of an early MRSA result compared to standard culture on 1) individual antibiotic therapy; 2) individual infection control (IC) measures; 3) financial cost of earlier interventions and; 4) bed management/nursing staff levels in our critical care areas. A technical evaluation of the technique on blood, vascular catheter tips and respiratory samples was performed.
Methods: During a 3-month period swabs, respiratory samples, vascular catheter tips and positive blood cultures taken in our critical care units were processed by standard culture (salt broth enrichment then chromogenic agar) and the Baclite method (2-hour selective broth incubation; extraction of organisms by immuno-magnetic separation; selective lysis with lysostaphin; measurement of adenylate kinase levels at baseline and 2 hours). Individual patient management, IC interventions, bed management and staffing levels were reviewed. The rapid results were not revealed to staff on the units.
Results: 226 patients (811 samples) were tested. Overall, 6.4% of patients were MRSA positive. Screening swabs confirmed a high negative predictive value (NPV) for the rapid method (⩾94%). The NPV for respiratory samples, line tips and blood cultures was high (⩾87%), but the positive predictive value was low (⩽11%). Rapid results would have allowed alteration of antibiotic therapy and instigation of IC procedures 48 hours earlier for 3 patients on the general ICU (1 patient requiring increased nursing requirements) thus reducing MRSA exposure rates by 13 patient-days. No further cases of MRSA colonisation were identified due to this additional exposure.
Conclusion: The Baclite flex MRSA detection method performed well on swabs from patients in critical care units. Low PPVs for respiratory, blood and line tip specimens would impede its usefulness for early clinical management of infection. IC interventions could be instigated up to 48 hours earlier using the rapid result, thus reducing the overall exposure of patients to MRSA. In a low incidence setting the expected benefit on MRSA transmission rates is difficult to quantify, although the cost of earlier intervention can be significant. The potential saving, estimated to be over US$14,000 for a single MRSA bacteraemia, needs to be considered in the context of overall expenditure.

P2129 MRSA screening – Comparison of chromogenic agar and enrichment broths

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Objective: The detection of meticillin-resistant *Staphylococcus aureus* (MRSA) in clinical samples is important. This is particularly so during screening in a low prevalence setting, where extensive infection control measures are imposed by a positive finding. The NaCl concentration is important for mecA expression, we therefore compared our routine chromogenic agar with two variants of enrichment broth one with 0.5% NaCl and another with 2% NaCl, during two outbreaks in nursing homes.
Methods: The chromogenic agar (bioMérieux) were compared with a modified enrichment broth originally described by Wertheim et al. with 0.5% NaCl concentration (broth1) and an another identical variant with 2% NaCl concentration (broth 2). Screening samples received from the nursing homes were inoculated on chromogenic and blood agar and in broth, MRSA isolates were verified by presence of mecA and Sa442. All isolates were characterised with MLST, spa-typing, SCCmec type and presence/absence of PVL. McNemar and Sign test were used for testing differences in detection rates.
Results: Broth 1 (0.5% NaCl); 4931 samples were included. MRSA were found in 187 samples 3.9% (95% CI; 3.3–4.3). The main results are presented in table 1. Broth 1 and chromogenic agar detected 17% (95% CI; 12–22) more MRSA-isolates than Chromogenic agar alone.

Table 1

Broth 1	Chromogenic agar	
	MRSA positive	MRSA negative
MRSA positive	83	32
MRSA negative	72	4744
		$P < 0.001$

Broth 2 (2% NaCl); 1697 samples were included. MRSA were found in 104 samples 6.1% (95% CI; 1.5–10). The main results are presented in table 2. Broth 2 and chromogenic agar detected 31% (95% CI; 22–40) extra MRSA than chromogenic agar alone and had the same sensitivity as the Chromogenic agar.

Table 2

Broth 2	Chromogenic agar	
	MRSA positive	MRSA negative
MRSA positive	41	32
MRSA negative	31	1583
		$P > 0.05$

All isolates belonging to the outbreaks were MLST 8, spa-type 304, SCCmec IV and PVL neg. Oxacillin MIC ranged from 1 to 64 mg/l. Three isolates with oxacillin MIC < 2 mg/l were CO_2 dependent and were detected on blood agar only.
Conclusion: The addition of an enrichment broth to chromogenic agar when screening for MRSA increases sensitivity significantly and 2% NaCl concentration seems better than 0.5%. MRSA isolates with low mecA expression (low Oxacillin MIC) were not detected by the selective media.

P2130 **Rapid detection of meticillin-resistant *Staphylococcus aureus* from nares and blood cultures using a new chromogenic agar**

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Objectives: Controlling the spread of meticillin resistant *S. aureus* (MRSA) has become a public health priority. While MRSA has long been recognised as a potential hospital associated pathogen, recent focus on more virulent strains of MRSA in the community has dramatically increased public awareness. Hospitals have been charged with the task of screening patients for MRSA colonisation and/or infection in order to prevent transmission in healthcare facilities and to institute appropriate therapy. Therefore we compared the performance of a new chromogenic screening agar, Spectra MRSA™ (SM), which selects for and identifies MRSA within 24 hours, to routine culture which can take 48–72 hours.
Methods: 120 nares swabs and 80 blood cultures positive for Gram-positive cocci in clusters (N = 80) were cultured on MRSA Spectra™ (Remel, Lenexa, KS) media as well as our routine battery which includes colistin nalidixic acid (CNA) agar for nares and a sheep blood/chocolate/MacConkey agar tri-plate for blood cultures (BD, Sparks, MD). MRSA appear as denim blue colonies on SM agar. Performance was evaluated based on recovery and time to identification. Identifications were confirmed by MicroScan (Dade, Sacramento, CA). Meticillin resistance was confirmed by PBP2′ latex agglutination and cefoxitin disk screen.
Results: Of the 120 nares swabs, 29 were culture positive for MRSA and all were detected on SM agar. In addition, 21 meticillin susceptible SA (MSSA) did not grow on SM agar. For the 80 blood cultures, 19 were culture positive for MRSA and all were detected on SM agar; 18 MSSA did not grow. Overall 98% of MRSA were detected within 24 hours of agar inoculation. The sensitivity, specificity, positive and negative predictive values for the SM media were 100%.
Conclusions: Rapid detection/reporting of MRSA from surveillance and clinical specimens is critical. Timely dissemination of this data enables appropriate infection control measures to be instituted and proper therapy or decolonisation protocols to be initiated. Utilisation of the Spectra MRSA agar would have resulted in almost all MRSA isolates to be reported within 24 hours. For small to mid-size laboratories that may not have the financial or technical resources to introduce molecular technology, this screening media is an excellent alternative for the rapid detection of MRSA. Microbiology laboratories should consider the use of this selective agar as part of MRSA identification algorithms.

P2131 **A novel chromogenic formulation for detecting meticillin-resistant *Staphylococcus aureus* in surveillance specimens: comparison to Oxoid Canada's Demin Blue agar**

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Objectives: Previous studies have shown Canada's Demin Blue (CDB) to be highly sensitive and specific, surpassing similar media in its cost-effectiveness and time to MRSA detection. Incubation at 37C enabled MRSA confirmation <24 h of plating, as CDB produced larger colonies earlier than other agars. As occasional Canadian MRSA have low cefoxitin (fox) MIC, this prospective blinded study aimed to further enhance our detection capabilities by evaluating a newly formulated medium without fox, the novel chromogenic formulation (NCF).
Methods: Consecutive MRSA swabs were tested in parallel on CDB and NCF, with plate order rotated to avoid inoculation bias. After incubation (~24 h x 37C in the dark), MRSA produced typical demin-blue colonies on both media. As in earlier studies, hazes, pinpoints and pale colonies were ignored and MRSA were confirmed directly from CDB and NCF if possible using Pastorex Staph Plus, tube coagulase, PBP2a and CLSI oxacillin screen. Discrepancy resolution entailed review of patient culture history, re-plating of the original swab to CDB and NCF, followed by broth enrichment and re-inoculation to both media. All MRSA were fully characterised.
Results: In total, 134 MRSA from 101/1,517 (6.7%) patients at 13 hospitals were grown from 2558 swabs: 65/1246 nares/axilla/groin/perineum (NAGP); 22/595 nasal; 21/542 rectal; 26/175 wound. 123 MRSA grew on CDB and 119 on NCF. There were 11 false-positives (FP) from 9 NAGP and 2 rectal swabs on CDB (7 coagulase-negative staphylococci (CNST), 2 *Bacillus* sp., 1 *Enterococcus*, 1 coliform) and 17 FP from 12 NAGP, 3 wound and 2 nasal swabs on NCF (12 CNST, 5 meticillin-susceptible SA). The overall % sensitivity, specificity, positive (PPV) and negative predictive values were 91.8, 99.6, 91.8, 99.6 for CDB and 89, 99.3, 87.5, 99.4 for NCF. MRSA typing identified 32 subtypes from 4 lineages: CMRSA2/USA100/spa2/ST5/SCCmecII-related MRSA were most prevalent followed by CMRSA10/USA300/spa1/ST8/SCCmecIVa/pvl+ community strains. No one MRSA type was associated with failure to grow on either agar; discrepancy analysis suggested detection failure was more likely due to low CFU/swab. The trend towards 18 h growth of MRSA on NCF was not significant in this sample size (P = 0.17).
Conclusions: The equivalence in sensitivity (P = 0.86) and specificity (P = 0.79) for CDB and NCF indicate that they could be interchangeable if MRSA with low fox MIC became prevalent. While highly specific, PPV of <92% reaffirms the need to prove MRSA from all new cases from any chromogenic media.

P2132 **Evaluation of the 3M BacLite Rapid MRSA test for the direct detection of MRSA from nasal, groin, and combined nasal/axilla/groin surveillance specimens**

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Background: The aim of this study was to determine the performance characteristics of the 3M™ BacLite™ Rapid MRSA culture based test for the detection of MRSA direct from screening swab taken from different body sites when compared to 48hour incubation with chromogenic agar.
Method: The analytical specificity and sensitivity of the assay was determined by challenging the assay with 0.5 Macfarland standard of stock cultures of a number of organisms:

- Historical wild strains of Ciprofloxacin resistant (5) and sensitive (11) MRSA diluted between 1:10 and 1:10000
- 10 different types of routinely isolated NCTC control strains at 1:100 dilution

The clinical performance of the assay was determined by testing 631 routine nasal (151), groin (148), and combined nasal/axilla/groin (332 NAG) swabs originated from Charing Cross and Chelsea & Westminster Hospitals. All results were compared to current operating procedures which consisted of inoculation onto Biomerieux MRSA ID chromogenic agar incubated aerobically at 37C for 40–48 hrs. Reference method plate cultures were inoculated first followed by the BacLite Rapid MRSA assay.
Results: Of the spiked MRSA samples there was only a marginal difference in sensitivity at very low colony forming units (2 or 3 at 1:1000 dilution). All strains grew on both systems. The 3 discrepant Ciprofloxacin Resistant strains were noted at the highest dilutions. Concordant results at 1:100 dilution were seen with both methods for both Ciprofloxacin sensitive and resistant wild strains. One false positive result seen in BacLite™ was noted with the non-MRSA *C. albicans* NCTC strain at 1:100 low dilution.
Of the 631 clinical specimens 151 were nasal swabs. 3M™ BacLite™ Rapid MRSA detected 146 of the 148 negatives giving a diagnostic specificity of 98.6% and a negative predictive value (NPV) of 100%. Of the 148 groin swabs 3M™ BacLite™ Rapid MRSA detected 127 out of 146 negatives, giving a diagnostic specificity of 87% and a NPV of 99.2%. For the 332 NAG swabs the diagnostic specificity was 82% (264/323) and NPV 99.2%. The low prevalence rate of MRSA at these 2 hospital sites (3.2%) meant that there was insufficient number of positives to report reliable performance data on diagnostics sensitivity from the swabs.
Conclusion: The high negative predictive value of the 3M™ BacLite™ Rapid MRSA test for nasal, groin, and NAG combined sites suggests that

the 3M™ BacLite™ Rapid MRSA test is a rapid and reliable screening test in low prevalence settings.

P2133 Cost-efficacy of a chromogenic agar for meticillin-resistant *Staphylococcus aureus* screening in a low-endemic setting

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Objectives: Screening and pre-emptive isolation of high-risk patients are key factors of the Dutch search and destroy strategy to control MRSA. However, the majority of high-risk patients are not colonised and remain in isolation for 3–5 days awaiting conventional culture results. In a multi-centre study we evaluated the chromogenic medium MRSA-ID (bioMérieux, Marcy-l'Etoile, France) for identification of MRSA in a low endemic setting and determined cost savings when isolation measures would have been based upon these results.
Methods: As part of a multi-centre study, high-risk patients for MRSA colonisation who fulfilled the criteria for pre-emptive isolation in 10 participating hospitals between 03/06 and 06/07 were eligible. In addition to the standard microbiological cultures, specimens were directly inoculated onto chromogenic media; results were read (based on morphology only) after 24 hours of incubation, however, did not influence decisions on isolation status.
Results: 711 patients were included (38% in the out-patient department), because of previous hospitalisation abroad (72%), contact with pigs (11%) and contact screening related to MRSA-positive patients (9%). A total of 2420 specimens were tested by chromogenic agar. The MRSA prevalence among these patients was 2.7%. Compared to conventional culture (including broth enrichment) sensitivity, specificity, positive predictive value and negative predictive value (NPV) of the chromogenic agar were 84.2%, 96.4%, 39.0%, and 99.6%, respectively.
Times from start of isolation up to notification of chromogenic agar or culture results were $30\frac{1}{2}$ hours and 92 hours, respectively. When isolation measures would have been discontinued upon negative chromogenic agar results, 94% (415/443) of hospitalised patients would have benefited and 821 isolation days would have been saved. There was 1 hospitalised patient with a false-negative chromogenic agar result. Costs of the chromogenic agar when added to conventional cultures were calculated €1.78 per test, yielding a cost per isolation day saved of €3.50.
Conclusion: Direct plating on chromogenic agar is suitable for MRSA screening in a low-endemicity setting with a NPV of 99.6%, potentially decreasing isolation time with $2\frac{1}{2}$ days per patient at the costs of €3.50 per isolation day saved.

P2134 A comparative evaluation of chromogenic culture media for the detection of meticillin-resistant *Staphylococcus aureus* from clinical specimens

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Objectives: Meticillin resistant *Staphylococcus aureus* (MRSA) is a major cause of nosocomial and community-acquired infection worldwide. Infection Control Teams and clinicians rely on accurate and timely results from patient screening to ensure appropriate control measures are implemented. Chromogenic culture media have been shown to have superior sensitivity and specificity in comparison with traditional culture methods for detecting MRSA from clinical samples. This evaluation was designed to examine the performance of a new medium from Oxoid (Brilliance™ MRSA) in comparison to the current Oxoid product (Chromogenic MRSA Agar), Becton Dickinson CHROMagar™ MRSA Agar and bioMérieux ChromID™ MRSA Agar.
Methods: Three hundred freshly collected swabs from a total of 99 patients were analysed in this study. Samples from sites commonly associated with carriage or colonisation (e.g. nasal, groin, axillae, wounds, intravenous lines and urinary catheter sites) were preferentially included. The swabs were emulsified in 3 ml sterile saline and vortexed. Each medium was inoculated with 10 microlitres of the sample suspension using the diminishing sweep technique. Results were recorded after 18–24 hours aerobic incubation at 35 to 37ºC. CHROMagar and ChromID plates showing negative results were re-incubated to 48 hours, in accordance with the manufacturers' recommendations.
Results: When combined results for all media were analysed, 269 of the 300 clinical samples were identified as negative for MRSA. Both Oxoid media correctly identified the highest number of samples positive for MRSA (28/31) within 24 hours, yielding a sensitivity of 90.3%. CHROMagar and ChromID required 48 hours incubation and both media yielded sensitivities of 83.9% (26/31). The highest specificity was also achieved by Oxoid Brilliance™ MRSA (97.4%), which yielded only 7 false positive results. Oxoid Chromogenic MRSA Agar gave 9 false positive results, providing a specificity of 96.7%. CHROMagar and ChromID yielded 30 and 33 false positives, providing specificities of 88.8% and 87.7%, respectively.
Conclusion: The new Oxoid Brilliance™ MRSA demonstrated comparable performance to Chromogenic MRSA Agar and achieved the best overall results within 24 hours. CHROMagar™ and ChromID™ required 48 hours incubation before all final results were obtained and demonstrated lower sensitivity and specificity. Oxoid Brilliance™ MRSA provided the best performance with the shortest incubation time.

P2135 Accuracy of SensiTitre microdilution panels in detecting oxacillin resistance in *Staphylococcus aureus* and coagulase-negative staphylococci

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Objectives: *Staphylococcus aureus* and coagulase negative staphylococci (CoNS) can be significant human pathogens. Oxacillin resistance in both groups of organisms has continued to expand and must be considered when treating staphylococcal infections. We prospectively evaluated SensiTitre microdilution panels incubated in the TREK Automated Reading and Incubation System (ARIS) for accuracy in detecting oxacillin resistance in clinical isolates of *S. aureus* and CoNS, with comparison to mecA PCR, and oxacillin supplemented agar.
Methods: Susceptibility testing was completed on 674 clinical isolates of *S. aureus* and 85 strains of CoNS using SensiTitre GPN2F microdilution panels. SensiTitre panels were auto-inoculated and then placed in the ARIS for incubation and auto-reading. All panels were read by the instrument after 24 hours of incubation; MICs were determined and interpreted using software and CLSI standards. *S. aureus* strains were also tested on Mueller-Hinton agar supplemented with 4% NaCl and 6.0 ug of oxacillin. Real-time PCR for detection of the mecA gene was performed on all isolates.
Results: Oxacillin resistance was confirmed in 324 (48%) and 53 (62%) isolates of *S. aureus* and CoNS respectively. Resistance was accurately detected with SensiTitre panels isolates, except one strain of CoNS; this isolate was reproducibly PCR positive for mecA, and repeatedly yielded an MIC of $\leqslant$0.25 ug/ml. For all 759 isolates, the MIC interpretation and the mecA PCR result were in agreement 98.4% of the time. False-negative mecA PCRs were noted in 12 instances involving 4 isolates of CoNS and 8 isolates of *S. aureus*. As expected, oxacillin agar was very reliable in detecting resistance in *S. aureus*; however, two resistant strains were not identified. Both had reproducible MICs of 4.0 ug/ml, and one isolate was also mecA PCR positive.
Conclusions: Susceptibility testing remains an important function in clinical microbiology, and laboratories increasingly rely on commercial systems to standardize and expedite this process. The inability of automated susceptibility testing systems to accurately detect oxacillin resistance in staphylococci has been reported, necessitating supplemental testing. This study evaluated the precision of the ARIS and SensiTitre microdilution panels in detecting oxacillin resistance. SensiTitre panels proved to be quite reliable in detecting resistance, and compared favourably with mecA PCR and oxacillin agar.

P2136 **Comparison of two chromogenic media for recovering vancomycin-resistant strains enterococci from stool samples**

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Objectives: Identifying of patients with gut colonisation by vancomycin-resistant strains of *Enterococcus faecalis* (VREfs) or *E. faecium* (VREfm) is of growing importance in order to minimize their transmission. Therefore, a reliable, easy-to-perform and cost-effective test procedure is needed. Several commercially available media had been introduced recently claiming to fulfil these demands. We here compare the performance of two of them.
Methods: For enrichment of VRE from stool from endangered patients approx. 20 mul was cultivated at 37º C overnight in a bhi broth (9 ml) with addition of one antibiotic disc containing 30 mug vancomycin. Then a subculture was performed on CHROMagar® (CHR; MAST Diagnostika) and ChromID VRE® (C-ID; bioMérieux). After 24 and 48 h of incubation at 37ºC the plates were evaluated for growth of suspicious colonies using the criteria given by the manufactures (rose–mauve colonies, bluish green or violet colonies; respectively). In case of tiny colonies a stereomicroscope was used. After performing a Gram stain of suspected VRE colonies they were further confirmed by susceptibility testing of furazolidone 50mug, mupirocin 10 mug (both Rosco), vancomycin, teicoplanin (both Etest®) and fermentation of D-xylose in order to rule out presence of intrinsic low-level vancomycin-resistant enterococci i. e. *E. casseliflavus* or *E. gallinarum*.
Results: Plating all type strains of the genus *Enterococcus* (n = 32) on both media revealed no growth whereas using VREfs (n = 1) and VREfc (n = 2) reference strains showed the described typical colonial appearance.
By screening of 116 stool samples so far we recovered 34 (29.3%) VREfm strains. All of them grow on both media after 24 h of cultivation and exhibiting a MIC $\geqslant$256 mg/l for vancomycin and $\geqslant$24 mg/l for teicoplanin consistent with the presence of a vanA gene. After 48 h of cultivation no growth could be revealed on 63 CHR plates in contrast to 48 in the case of C-ID. Microbes other than VRE cultivated are yeasts, Gram-negative and Gram-positive bacteria not showing the typical colonial appearance of VRE. Evaluation of C-ID plates was somehow easier than that of CHR because tiny colonies on the latter one could be not so easily detected due to minor contrast caused by the milky media.
Conclusion: Both chromogenic media tested could be used for detecting of VRE from stool samples with a high specificity. A prior selective enrichment culture avoids unnecessary workload caused by growth of other microorganism.

Borreliosis

P2137 **Co-infection with *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, *Bartonella* sp. and *Babesia microti* in patients suspected of Lyme disease**

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The aim of this study was to determine coinfection with 4 different infectious agents in patients suspected of Lyme disease.
Sera collected from 110 patients and 50 blood donors were examined with ELISA IgM and ELISA IgG for antibodies to *B. burgdorferi*. Also antibodies to *A. phagocytophilum*, *Bartonella* sp. and *B. microti* were detected with specific IgG immunofluorescence assay kits.
Antibodies for *B. burgdorferi* were found in 65 (59.1%) of suspected patients. In 82 (74.5%) patients antibodies for *Bartonella* sp. were found, and antibodies for *B. microti* and *A. phagocytophilum* were detected in 31 (28.2%) and 23 (20.9%) patients, respectively.
No serum without any antibodies was found. In 35 sera only one kind of antibodies was found. In 75 sera coinfection was noted (60 sera with 2 agents, 14 sera with 3 agents, and 1 serum with all for agents).
In 41 patients (37.3%) the most common coinfection of *B. burgdorferi* and *Bartonella* sp. was evidenced (in 32 cases with these two agents, in 5 cases with additional *B. microti*, in 3 cases with additional *A. phagocytophilum*, and in one case where all 4 agents were detected). In the control group there was found 12 positive results (24%) for *Bartonella* sp., 4 (8%) for *B. burgdorferi*, 3 (6%) for *A. phagocytophilum*, 0 positive results for *B. microti*. In one case coinfection was determined (*Bartonella* sp. and *A. phagocytophilum*).
These results prove that coinfection is common in patients suspected of Lyme disease and laboratory testing for coinfection is necessary to establish exact diagnosis and treatment.

P2138 **Evaluation of a CLIA test for detection of *Borrelia burgdorferi* specific IgM-antibody production in cerebrospinal fluid**

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Objective: Detection of *Borrelia burgdorferi* specific antibody synthesis in cerebrospinal fluid (CSF) by calculation of the *Borrelia* specific antibody index (AI) is of paramount importance for diagnosis of neuroborreliosis. Combined with unspecific CSF findings – lymphocytic pleocytosis, elevated protein, CSF/blood barrier dysfunction – a reliable diagnosis can be achieved.
VlsE is an immunodominant antigen for serological diagnosis in early and late manifestations of Lyme borreliosis including neuroborreliosis. It was recently demonstrated to be a powerful tool for increasing sensitivity of IgG-immunoassays for detection of *Borrelia* antibodies in serum as well as for determination of the IgG-AI. By contrast, studies on IgM-AI determination are rare so far. Therefore, we studied performance of a new chemiluminescent (CLIA) *Borrelia* IgM assay (LIAISON® *Borrelia* IgM Quant, DiaSorin), using recombinant VlsE and OspC for *Borrelia* specific intrathecal antibody production in comparison to an evaluated in-house ELISA based on a *B. afzelii* strain PKo ultrasonicate.
Methods: We studied 31 paired serum/CSF samples collected from patients with well defined stage II neuroborreliosis. A prospective panel included 218 consecutive paired serum/CSF samples collected from subjects investigated for neuroborreliosis. Whenever possible the AI was calculated by the method of Reiber.
Results: 29 out of 31 samples collected from patients with neuroborreliosis had measurable *Borrelia* IgM antibodies in CSF samples by the CLIA test. Calculation of the AI resulted in 23 positive, 1 equivocal and 5 negative (diagnostic sensitivity was 23/31, corresponding to 74.2%). Out of the 31 samples 22 had measurable *Borrelia* IgM antibodies in CSF samples by the in-house test. The calculated AI was positive in 20, equivocal in 1 and negative in 1 (diagnostic sensitivity was 20/31, corresponding to 64.5%).
Out of the 218 consecutive CSF samples 211 and 208 were negative by the in-house and the CLIA method, respectively. 7 AI were concordant positive with both methods while 3 CSF samples had measurable antibodies by CLIA only. The AI of those 3 samples were negative, equivocal, and positive in one each. The positive sample showed also a positive AI for IgG.
Conclusion: The results indicate that the fully automated CLIA *Borrelia* IgM assay is a reliable test for detection of *Borrelia*-specific intrathecal IgM antibody production that may add significantly to the diagnosis of early neuroborreliosis.

P2139 **Nested-PCR in the diagnostic of different forms of Lyme borreliosis**

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Objectives: The newly derived targets specific for *Borrelia burgdorferi* sensu lato were tested in the group of patients with different clinical forms of Lyme borreliosis. Nested-PCR system was used and five targets were applied in parallel. Sensitivity and clinical utilisation of PCR were evaluated.
Methods: 124 patients were enrolled into the prospectively designed study – 53 patients suffered from neuroborreliosis, 23 patients had

lyme arthritis and acute erythema migrans had 48 patients. Diagnosis was established on the typical clinical symptoms and proof of specific antibodies in serum, and/or in CSF in neuroborreliosis, and in synovial fluid in arthritis. Five targets were tested in parallel – specific for 16S rDNA, flagellin, p66, OspA and OspC proteins. DNA was examined in plasma and urine in all patients. Moreover, CSF in neuroborreliosis and synovial fluid in arthritis were examined before treatment.
Results: 41 patients (77.4%) were positive for neuroborreliosis, 27 DNA positive patients (56.3%) were found in the group with skin involvement and 13 patients (56.5%) were DNA positive in group with arthritis. The specific DNA was found in 50% of patients with neuroborreliosis (22 patients) after treatment, in 7 patients with erythema migrans and in 10 patients with lyme arthritis. After 3 months the DNA positivity was found in 29 patients (29.0%) and after 6 months in 7 patients (9.0%). The highest frequency of positive amplifications showed the primer specific for 16S rDNA (38.7%) and the lowest one for p66 protein (positive only 11 times – 4.4%). By using one primer (16S rDNA) only 48 positive patients (38.7%) would have been diagnosed with two parallel amplifications 65 positive patients (52.4%) would have been found. Three parallel amplifications would dicover 74 (59.7%) patients.
Conclusion: The nested-PCR method achieved before treatment in all clinical forms of Lyme borreliosis the general sensitivity 65.3% (81 positive patients out of 124) by using five parallel amplification. The highest sensitivity was found in the sequence specific for 16S rDNA (38.7% of all positive amplifications) the using of two or three parallel amplification is suitable to obtain the sufficient clinical sensitivity. Sensitivity of primers didn't differ in all clinical groups of borreliosis.
Study was supported by public government grants CR IGA8293, MSM 0021620812.

P2140 Comparison of automated immunoassay LIAISON® *Borrelia* and ELISA Enzygnost® Borreliosi for the diagnosis of Lyme disease

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Objectives: Lyme borreliosis is multisystem diseases caused by the tick-borne spirochetes belonging to the species *Borrelia burgdorferi* sensu lato. The diagnosis of Lyme disease is based on clinical manifestations and laboratory findings. The serological detection of *Borrelia* antibodies represents a stone corner for diagnosis. Aim of the present study was to evaluate the diagnostic performances of automated immunoassay LIAISON® *Borrelia* IgG and IgM antibodies (DiaSorin – Italy) to *Borrelia burgdorferi* in comparison with ELISA Enzygnost® Borreliosi IgG and IgM (Dade Behring – Germany).
Methods: Study group: 256 serum specimens from patients with clinically characterised Lyme borreliosis at different stages. All patients came from an endemic area in the North East of Italy. All serum specimens were tested in parallel with Enzygnost® Lyme LinkVlsE IgG and Borreliosi IgM (Dade Behring-Germany) and with LIAISON® *Borrelia* IgG and LIAISON® *Borrelia* IgM Quant (DiaSorin-Italy).
Enzygnost® Borreliosi IgG and IgM are immunoenzymatic assays based on a mixture of inactivated *Borrelia burgdorferi* antigens for qualitative IgG and IgM determination in serum/plasma.
LIAISON® *Borrelia* IgG and LIAISON® *Borrelia* IgM Quant are chemiluminescent (CLIA) fully automated assays for quantitative determination of specific antibodies IgG or IgM in serum/plasma and CSF. Recombinant VlsE antigen is used for coating magnetic particles in the IgG assay. Two recombinant antigens OspC and VlsE are used for coating magnetic particles in the IgM assay. When the sample was positive for IgG and/or IgM the specimens was graded reactive. The discordant samples were solved with immunoblot assay.
Results: The diagnostic sensitivity was determined by testing 256 serum specimens from patients with clinically characterised Lyme borreliosis at different stages (Eythema migrans, Neuroborreliosis, Arthritis). The percent IgG and/or IgM positive sera was 93% for Enzygnost® Lyme LinkVlsE IgG and Borreliosi IgM and 97% for LIAISON® *Borrelia* IgG and LIAISON® *Borrelia* IgM Quant.: nº4 Neuroborreliosis sera are positive for IgG and IgM LIAISON® *Borrelia*, Enzygnost® IgG positive and Enzygnost® IgM negative.
Conclusions: These preliminary data show a good diagnostic sensitivity of LIAISON® *Borrelia* IgG and LIAISON® *Borrelia* IgM Quant. Further study are going to evaluate the diagnostic specificity by testing 250 routine serum specimen from subjects living in non endemic area with all assays.

P2141 Recombinant *Borrelia burgdorferi* protein antigens in the serology of early Lyme borreliosis

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Objectives: Early diagnosis and treatment of Lyme borreliosis are of particular interest to prevent late untreatable manifestations of the disease. OspC, FlaB, DbpA, and VlsE proteins of *B. burgdorferi* are known to be among the limited number of antigens that induce immune response in early Lyme borreliosis.
Methods: OspC, flab, dbpA, and vlsE genes were amplified from genomic DNA of *B. burgdorferi* and cloned in *E. coli*. Specificity of the obtained proteins was confirmed by specific monoclonal antibodies in immunoblot. Recombinant ELISAs with each protein as a single antigen were developed and a serum panel from 50 patients with clinically defined early Lyme borreliosis (erytema migrans) was tested. Serum samples from 10 healthy individuals served to calculate the cut-off. A serum panel, consisting of sera from 10 patients with syphilis and 10 patients with rheumatoid arthritis, both groups known to give cross-reactivity, was used to test the assays specificity.
Results: OspC and VlsE ELISAs showed the best balance between sensitivity and specificity.
Conclusions: Neither antigen was enough sensitive and specific to be used in a single antigen based ELISA.

P2142 Quantification of the synthesis of antibodies against *Borrelia burgdorferi*-specific antigens within the central nervous system by chemiluminescent and enzyme immunoassay

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Objectives: Diagnosis of central nervous system (CNS) involvement of infections by *Borrelia burgdorferi* sensu lato may be difficult when the clinical syndrome is atypical or when the interval between infection and cerebrospinal fluid (CSF) analysis is long. Diagnostic analysis of the CSF is complicated by antigenetic heterogeneity of *Borrelia burgdorferi* strains and differences in antigen compositions of diagnostic tests. This study compared the ability of chemiluminescent (CLIA) and enzyme immunoassay (EIA) to detect antibody synthesis directed against *Borrelia burgdorferi* sensu lato within the CNS.
Methods: Antibodies against *Borrelia burgdorferi* in CSF and serum pairs from 90 patients were quantified by CLIA (LIAISON BORRELIA, Diasorin, Saluggia, Italy) or by EIA (in-house method using ENZYG-NOST Lyme Link VlsE plates, Dade Behring, Schwalbach, Germany). The *Borrelia*-specific IgG and IgM antibody index (AIIgG, AIIgM) was calculated: (*Borrelia*-specific Ig in CSF/*Borrelia*-specific Ig in serum)/(total Ig in CSF//total Ig in serum).
An AI > 1.5 was considered abnormal.
Results: In 47 cases, an elevated AI either for IgG or IgM or both suggesting *Borrelia*-specific antibody synthesis within the CNS was detected both by CLIA and EIA. The absolute AI values, however, showed considerable deviation among both methods. In 39 cases, AIIgG and AIIgM were normal or the *Borrelia*-specific CSF antibody concentration of IgG, IgM or both was below the quantification limit. In 3 cases, EIA detected IgG or IgM synthesis in CSF, whereas the AIs determined by CLIA were normal. In one case, AIIgM determined by CLIA was elevated, but below the detection limit when measured by EIA.
When *Borrelia*-specific IgG and IgM were analysed separately, the rate of incongruent results between the two methods was higher. In 29

cases, AIIgG was normal as determined by EIA, but not measurable by IML. In 5 cases with normal AIIgG determined by EIA, CLIA yielded implausibly low AIIgG (<0.5). In 11 cases, AIIgM was not detectable by EIA, but elevated when measured by CLIA.
Conclusion: CLIA was more sensitive than EIA in detecting *Borrelia*-specific IgM synthesis. EIA was more sensitive to detect *Borrelia*-specific IgG in CSF of patients with an elevated or normal AIIgG.

P2143 A cell envelope protein array of *Borrelia burgdorferi* to profile the humoral response of patients with Lyme disease

Y. Xu, J. Bruno, B. Luft (Stony Brook, US)

Objective: To determine the cell envelope proteins of *Borrelia burgdorferi* recognised by immune sera of patients with late Lyme disease.
Methods: We developed a *Borrelia* microarray containing proteins encoded by 90 cell envelope genes and their homologs described in the annotated genomic sequence of *B. burgdorferi*, strain B31. The protein microarray was used to profile the humoral immune response using sera from 14 individuals with late Lyme disease and 4 negative controls.
Results: Although there were sera-specific responses, 15 of the cell-envelope proteins were recognised by 9 or more samples. Sera from non-infected individuals lacked reactivity against any of the proteins on the array. Among the most antigenic envelope proteins, protein sequence analysis using the BLASTx programme of GenBank revealed little homology to known microbial proteins from other species. Seven of the 15 highly antigenic proteins were hypervariable with multiple homologs found in our genomic analyses of various strains in the species. Approximately one half of the proteins that were highly antigenic in our array analysis were not found in *Borrelia* cultured in vitro. The proteins that were highly seropositive included BBA24 (decorin binding protein A (dbpA))(12 out of 14 samples), and paralogous family gbb fam_PF05714 (immunogenic protein P35) (9 of 14 samples).
Conclusions: The accurate diagnosis of Lyme disease depends on correlating objective clinical abnormalities with serological evidence of exposure to *B. burgdorferi*. A protein array of the envelope proteins of *Borrelia burgdorferi* may be very useful in specifically identifying patients with Lyme disease. This approach could contribute to a more rapid discovery of antigens not expressed in vitro which may be useful for the development of vaccine and diagnostics.

P2144 Protein expression of *Borrelia burgdorferi* sensu lato strains during long-term in vitro cultivation

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Objective: To establish whether and how long-term cultivation influences the expression of borrelial flagellin, proteins in the range of heat shock proteins (Hsps) and in outer surface proteins (Osps), i.e. proteins known to be involved in pathogenesis of and immune response to borrelia infection.
Material and Methods: We analysed 24 *Borrelia* strains (8 *B. afzelii*, 6 *B. garinii*, and 10 *B. burgdorferi* sensu stricto) Strains were cultivated in MKP medium and passages were performed in the exponential phase of growth. 20 passages of each strain were performed during a period of approximately 1 year and five protein profiles were determined: the initial immediately after establishing positive culture result, the second after 5th, the third after 10th, the fourth after 15th and the last after 20th passage. Protein profile was performed using sodium dodecyl sulfat polyacrilamid electrophoresis (SDS-PAGE). The presence and the quantity of the expressed proteins were assessed and compared within individual strain.
Results: Hsps proteins and flagellin were consistently expressed in all analysed strains throughout the entire cultivation period, while pronounced differences in the expression of Osps were found. In some strains changes from expression to weak expression or even to non expression and to re-expression were established. During entire cultivation period only 5/24 (21%) isolates remained unchanged while in 19/24 (79%) isolates distinctions in Osps were established: after 5th passage in 8 (33%) isolates, after 10th in additional 7 (29%) isolates, after 15th in 3 (13%) and after 20th passage in 1 (4%) additional isolate. Most often (in 16/24 isolates – 67%) alterations in expression of OspC occurred, followed by variations in OspB (10/24 – 42%) and OspA (7/24 – 29%). In 4 isolates changes of all three Osps occurred, in 6 isolates variations in two Osps were found (in 3 OspA and OspB, in other 3 OspB and OspC) while in 9 isolates solely changes in the expression of OspC were established. The most pronounced variations were ascertained among *B. afzelii* isolates, while in the other two *Borrelia* species the changes in the expression of Osps in the course of cultivation were less frequent.
Conclusion: During long-term borrelia cultivation Hsps proteins and flagellin are consistently expressed, while pronounced differences in the expression of Osps (most notably OspC) occur. The most prominent variations are present among *B. afzelii* isolates.

P2145 Seroprevalence of *Borrelia* infection among tick-bitten asymptomatic teenagers in risk region of southern Podlasie, Poland

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Objectives: The annual number of Lyme borreliosis (LB) cases in Poland shows a growing tendency and was amounted to 6680 in 2006. The highest incidence of LB is reported from Podlasie (north-eastern Poland), which is an endemic area and the rate reached the value of 77.2 per 100,000. The aim of the study was to educate and verify the knowledge about preventive methods reducing risk for infection as well as to evaluate the level of tick bites and the seroprevalence of antibodies against *Borrelia burgdorferi* in healthy teenagers bitten by tick.
Methods: The first stage of the study included 1044 healthy pupils from southern Podlasie region and level of tick bites was assessed. The levels of IgM and IgG antibodies against *B. burgdorferi* were determined in selected 85 (aged 16–18) bitten pupils by means of ELISA immunoenzymatic method, *Borrelia* 14 kD + OspC IgM ELISA and *Borrelia* IgG + VlsE ELISA (DRG Diagnostics, Germany) and confirmed with *Borrelia* Blot IgM and IgG. All participants were examined at the final period of tick activity (September 2007).
Results: Among teenagers 49.9 p.c. (n = 521/1044) were bitten by tick, 14.4 p.c. (n = 150/1044) more than twice. Positive results for IgM *B. burgdorferi* were revealed in 16.5 p.c. (n = 14/85) and 4 were doubtful, and for IgG, 3.5 p.c. (n = 3/85) individuals were positive. Detection with *Borrelia* Blot IgG (DRG) confirmed the presence of antibodies anty-VIsE and others specific protein. In two cases IgG antibody levels were extremely high (165.6 and 54.7 U/ml).
Conclusion: High percent of tick bitten teenagers and positive results of antibody levels confirm necessity of education of how to reduce the risk for infection, including daily self-examination for ticks, selective use of acaricides and tick repellents, use of practices that reduce tick populations in forest and tourist relaxation spots. Positive results for *B. burgdorferi* antibodies in asymptomatic period may change serological picture in case of the future clinical manifestation of borreliosis.

P2146 Study on the occurrence of *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, *Babesia microti* and *Bartonella* sp. in ticks from the Roztocze National Park (eastern Poland)

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The objective of the study was to evaluate the risk of acquring infection of *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, *Babesia microti* and *Bartonella* sp. for workers and visitors of Roztocze National Park (Eastern Poland).
Material and Methods: 606 *Ixodes ricinus* ticks (females, males and nymphs) collected from forest areas of Roztocze National Park (RNP) were examined for the presence of *Borrelia burgdorferi* sensu lato

(B.b.s.l.), *Anaplasma phagocytophilum*, *Babesia microti* and *Bartonella* sp by PCR method.
Results: The total infection rate of Bbsl, *A. phagocytophilum*, *B. microti* and *Bartonella* sp amounted: 11.6%, 6.1%, 0.5% and 2.2% respectively. Adult ticks were infected by these pathogens in the greater proportion than nymphs. In 6.5% of infected ticks coinfections with B.b.s.l. and *A. phagocytophilum* were observed.
Conclusion: The results proved that four pathogens transmitted by ticks are present in the forest environment of RNP and indicate a risk for the workers of RNP as well as for visitors to acquire infections caused by these pathogens, especially by *Borrelia burgdorferi* and *Anaplasma phagocytophilum*.

P2147 A high-virulence clone of *Borrelia burgdorferi* sensu stricto is distributed widely in Europe and North America

Y. Xu, J. Bruno, B. Luft (Stony Brook, US)

Objective: Among the multiple *Borrelia burgdorferi* species causing Lyme disease, only *B. burgdorferi* sensu stricto is found in both North America and Europe. We investigated the evolutionary link between the European and North American populations of *B. burgdorferi* sensu stricto by comparing the DNA sequences of isolates from the United States.
Methods: We sequenced one chromosomal locus (16S-23S ribosomal RNA intergenic spacer, IGS) and three highly variable plasmid loci (ospC on cp26, dbpA on lp54, and BBD14 on lp17) from U.S. (n = 36) and European (n = 32) isolates. Cluster analysis of multilocus sequences revealed 19 intra-specific genomic groups, 16 of which were strictly endemic lineages. One genomic group, the B31 clonal type, is the most frequent group in both continents. One single-nucleotide substitution and one gene-conversion over a span of 3,800 nucleotides distinguished the European and North American B31-type isolates. Sequence variations of the outer surface protein gene (ospC) were associated with the divergence of incipient genomic groups in both continents, corroborating the lineage-defining role of ospC variations. Linkage breakdowns between ospC and genomic groups were observed in one European isolate and 50% of the midwestern U.S. isolates, suggesting stable population sizes in those regions, in contrast to the rapid clonal diversification in the northeastern U.S.
Conclusions: We conclude that the bi-continental distribution of *B. burgdorferi* sensu stricto is due to post-glacial vicariance, indigenous diversification, and accelerated migration in modern times. The broad host- and vector-species range of a small number of intra-specific lineages (e.g., the B31 type) may underline their high pathogenecity in humans, and the introduction and preferential growth of these clones may have played a role in the rapid emergence of Lyme disease in the United States in recent decades.

Sexually transmitted diseases and infections of female genital organs

P2148 Oncogenic HPV subtypes in HIV-positive women

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Background: Women with HIV, especially those with low CD4 counts and high HIV viral loads, are more likely to have persistent genital oncogenic human papillomavirus infection and to develop cervical cytological abnormalities and neoplasia. HPV 16 and 18 are the most common oncogenic subtypes in the European population. African women are more likely to have non 16/18 subtypes.
As part of the Irish Cervical Screening Research Consortium, we are performing more intensive screening on HIV + patients to determine the effect of ART on HPV subtypes and cytology.
Method: All female patients attending the HIV clinic are being prospectively recruited into the study, with cervical cytology samples taken at baseline and regularly during follow-up.
The presence of HPV DNA was analysed using proprietary Hybrid Capture® 2 (hc2) signal-amplification (Digene) and PreTect HPV-Proofer was used to identify the 5 high-risk types 16, 18, 31, 33 and 45.
Results: To date, 91 women have been screened at baseline. Of these 48/91 (52%) were on ART. Only 7/91(8%) had a CD4 count $<200\times10^6$/L.
Oncogenic HPV subtypes were detected in a total of 15/91 (16%) women. 10/15 (64%) had non-16/18 subtypes. 8/15 had HPV 45, and 4/15 had HPV 33. 4/15 had more than one subtype. 7/15 of women eith oncogenic HPV were receiving ART, all of whom had a viral load <50 cpm. 1/15 (7%) had a CD4 count of $<200\times10^6$/L.
Cytological abnormalities (6 baseline nuclear abnormalities, 12 CIN1, 2 CIN2, 1 CIN3) were detected in 21 women, of whom 6 had oncogenic HPV infection detected on this occasion. Women with oncogenic HPV were more likely to have cytological abnormalities (OR 2.71, $p = 0.08$). Of the 21 with cytological abnormalities, 8/21 were on ART and 7/21 had a CD4 count of $<200\times10^6$/L. The odds ratio of having cytological abnormalities in those with CD4 counts $<200\times10^6$/L was 3.67 ($p = 0.13$).
Conclusion: More than half of the oncogenic HPV subtypes detected in this population were not 16 or 18, which has implications for vaccine use in women with HIV. Although oncogenic HPV infection was associated with a higher risk of cytological abnormalities, the majority of women with cytological abnormalities did not have HPV detected in this occasion. Further research will determine the suitability of using HPV detection in cervical screening.
Oncogenic HPV and/or cytological abnormalities was found in 22/84 (26%) women with high CD4 counts highlighting the importance of continuing cervical screening in this population.

P2149 Novel methods for the diagnosis of HPV infection

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Introduction: Human Papilloma Virus (HPV) infection is more common among HIV positive women. HPV-associated lesions are more frequent, more aggressive, and less responsive to therapy when compared to seronegative women. Classic methods (gynaecological examination, transvaginal US-scan, colposcopy and Pap test) in association with molecular test such as Hybrid Capture 2 (HC-2, Digene – Italy) are employed for diagnosis of HPV infection in HIV seronegative, and seropositive women. The purpose of this work is to evaluate the use of Pretect HPV Proofer for the detection of E6/E7 mRNA expression also in seropositive women.
Materials and Methods: We considered 25 HIV positive women admitted at the Clinic of Infectious Diseases of "G. d'Annunzio" University in Chieti. All women were evaluated with gynaecological examination, transvaginal US-scan and colposcopy. We have also used cytological techniques (Pap test) and molecular test (HC-2, and Pretect HPV Proofer).
Results: LSIL has been found in 28% of HIV positive women and HSIL in 8%. Abnormal cytology was more frequent among women with HIV viral load between 400 and 10.000 copies/ml (25% ASCUS, 42% LSIL e 100% HSIL), and lower CD4+ lymphocyte levels. Using HC-2 test HR-HPV genotype has been detected in 40% of women; 12% of women showed a positivity for LR, and HR-HPV infection. HPV infection is more common among women with high HIV viral load ($p = 0.001$; $r = 0.62$) and CD4 lymphocyte count below 500 cells/ml. A negative result for mRNA test was found in 68% of HIV+ women. The diagnosis of ANTZ was more frequent when HIV viral load was >10,000 copies/ml ($p = 0.04$; $r = 0.43$).
Discussion: HIV+ women are at higher risk for HR-HPV infection than uninfected women. This risk seem associated with high HIV viral load and low CD4 lymphocyte count. The role of HAART on HPV

infection evolution toward cervical dysplasia and cancer is somewhat unclear. Molecular techniques for HPV diagnosis and typing are more specific and accurate, when compared with other methods. In HIV+ women PreTect HPV Proofer is a promsing new tool for the diagnosis of HPV infection, for identification high risk HPV genotypes, allowing a more reliable follow up of HIV-HPV coinfection.

P2150 Demographic and epidemiological factors associated to *Treponema pallidum* infection in pregnant women

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Sexually transmitted infections (STI) constitute a pandemic with an estimated 15 millions persons acquiring a new STI each year. The possible resurgence of infectious syphilis, a disease previously believed to be close to eradication, is a matter of increasing global concern. Untreated cases of syphilis in pregnant women has been associated with adverse pregnancy outcome and serious health effects in the foetus and the newborn.

Objective: To identify demographic and epidemiological factors associated with syphilis in pregnant women.

Methods: A total of 27 consecutive pregnant women complicated with syphilis were studied, in eight the diagnosis was made with the physical finding of a genital chancre and a positive FTA-ABS test; in the rest, the diagnosis was suspected with a positive VDRL, and confirmed by means of the positively of the FTA-ABS test. For each pregnant woman with syphilis, four apparently healthy women were included as comparison group. Demographic, epidemiologic and obstetric characteristics of the two groups of patients were compared. Categorical data were analysed by Chi-square test or Fisher exact test; the Student's t-test was performed to analyse numerical data with a normal distribution. The strength of association was calculated with Odd Ratios (OR) with 95% confidence interval (CI). A multivariate analysis was made by means of logistic regression.

Results: The mean age of the patients was 24.2 ±7.4 years, and the mean age of begin sexual activity was 17.5 ±3.3 years. Eleven (40.7%) presented their first pregnancy. The variables that were associated with maternal syphilis during the gestation were: age less than 29 years, begin the sexual activity before 19 years, not to be married, multiple sexual partners, six or less years of formal education, and didn't have prenatal care. The logistic regression analysis showed that the variables independently associated with syphilis in pregnant women were six or less years of formal education and the marital status of not married.

Conclusion: The principals finding in the patients of this study was a low schooling, and an instable marital status with a high percentage of pregnant women not married who were single or live in free union, as the factors strongly associated to syphilis during pregnancy.

P2151 Incidence of syphilis in a cohort of HIV-infected subjects, Italy

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Background and Objective: Syphilis is a reliable marker of behaviours at increased risk for transmission/acquisition of sexually transmitted infections (STI) and HIV. Among HIV infected persons the incidence of syphilis reflects the potential for further spreading of the HIV virus in the community. We measured the incidence of syphilis in a cohort of HIV infected persons and described association with demographic and behavioural variables.

Methods: Retrospective, cohort analysis. All consecutive HIV infected persons registered at the Institute of Infectious and Tropical Diseases, Spedali Civili, Brescia from 1 January 2001 to 30 June 2007 were included. The Institute did not adopt specific strategies to reduce the risk of acquiring /transmitting STIs during the study period. All demographic, behavioural, and syphilis treponemal tests (TPHA) results were collected from clinical charts.

Results: Out of 1905 new HIV infected subjects enrolled during the study period 1280 (67.2%) had at least one TPHA test. A positive syphilis serology at the first test was documented in 238 subjects (18.6%). Of the 1042 remainder subjects 509 (48.8%) had at least one repeated test and were included in the retrospective cohort analysis. 47 (9%) seroconverters were identified over a mean follow-up period of 759 days (SD±668 days). Syphilis incidence was 43.8/1000 person/year (95% CI: 32.0–57.9). At univariate analysis, being male (OR 5.22, 95% CI: 1.84–14.81, $p < 0.005$) and homo/bisexual (OR 6.24, 95% CI: 3.32–11.72, $p < 0.00$) were associated with a higher risk of new syphilis infection. Nor age neither virological/immunological parameters were associated with the risk of syphilis. The Cox regression analysis showed that homo/bisexuality was independently associated with an increased risk of syphilis (HR 4.39, 95% CI: 2.33–8.30, $p < 0.005$).

Comments: In the absence of specific risk reduction programmes the incidence of syphilis remains high among HIV infected homo/bisexual males in Northern Italy. These data document the potential for continuous spread of HIV infection and other STI in this population.

P2152 Audit of laboratory diagnostic methods for syphilis in England and Wales

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Objectives: The number of cases of infectious syphilis is increasing rapidly across England and Wales (E&W). Both clinicians and microbiologists have expressed concerns about delays in providing patients with timely results and the potential impact on patient care. A standard operating procedure (SOP) for the serological diagnosis of syphilis has recently been developed by the Health Protection Agency (HPA) and ratified. Our objectives were to describe syphilis testing in primary diagnostic laboratories including timeline of result reporting, to describe the referral process to regional and reference laboratories, and to describe the experience of users.

Methods: A web-based questionnaire was designed, which incorporated feedback from an initial consultation process. All microbiology departments, genito-urinary medicine (GUM) clinics, and antenatal clinics in E&W were asked to complete this questionnaire. A pilot study was performed in London prior to national roll-out.

Results: Responses were obtained from 116/181 microbiology departments, 119/172 GUM clinics, and 169/199 antenatal clinics. The proportion of microbiology departments performing EIA, TPPA/TPHA, RPR/VDRL, and EIA IgM were 93.7%, 34.7%, 41.1%, and 10.5% respectively. Dark ground microscopy was performed by 34.5% of GUM clinics. 81% of microbiology departments reported a turnaround time for negative results of <1 week. There was a large variation in turnaround times for positive results (range <1 week to 6–8 weeks), with 18.5% of GUM clinics reporting turnaround times of >3 weeks. Delays in turnaround time which were reported to have had an adverse effect on patient management were reported by 25.2% of GUM clinics and 5.3% of antenatal clinics.

Conclusion: Syphilis testing and delays in providing results vary across E&W. To limit the spread of infection, turnaround times for the diagnosis of syphilis need to be significantly reduced.

P2153 Re-emergence of infectious syphilis in Constanta county, Romania

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Objectives: To evidence the re emergence of infectious syphilis in Constanta County, by testing the patients hospitalised in the wards of clinical hospital in Constanta (other than dermato-venerology department) for *Treponema pallidum*.

Methods: Serum samples were taken from 10712 patients admitted in Clinical Hospital (internal medicine ward, obstetrics, cardiology, surgery, neurology, paediatrics, intensive care unit and psychiatry). Cerebrospinal

fluid was collected from 4 patients(3 new born babies and one patient in neurology).
The used tests were RPR (Rapid Plasma Reagin test) and TPHA(*Treponema pallidum* haemagglutination test).
Results: Positive results were obtained in 320 cases representing 2.98% of investigated patients(42 males and 178 women, 210 adults and 10 children)
All 4 cerebrospinal fluid samples were positive for *Treponema pallidum*. We detected a number of 216 pregnant women and 8 new born babies which have been treated according to the therapeutical protocols.
Conclusion: A greater vigilance and screening for syphilis in population are needed, mainly among pregnant women and newborns. Thus we will be able to decrease the incidence of syphilis which is rising among women in Romania.
Control methods should be based on ante natal screening in the first trimester, supported by treatment and partner notification. These control methods are highly cost effective but depends on well structured healthcare system. Congenital syphilis is a preventable disease and its re emergence reflects a failure in syphilis control programmes.

P2154 Prevalence of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in low-risk and high-risk female population in Novosibirsk, Russia

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Objectives: Epidemiology of sexually transmitted diseases (STDs) is largely based on surveillance data. The aim of the present study was to examine the prevalence of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections in low-risk and high-risk groups among young women in Novosibirsk, Western Siberia.
Methods: We examined female university students, gynaecology clinic attendees, medical workers, and pregnant women. Overall, 439 women voluntarily participated in the study. After a confidential interview, a gynaecologic examination was performed with collection of endocervical specimens. Gen-Probe Pace 2 and APTIMA COMBO 2 Assay (Gen-Probe Incorporated, San Diego, CA, USA) were used for *N. gonorrhoeae* and *C. trachomatis* testing.
Results: The prevalence of *C. trachomatis* and *N. gonorrhoeae* infections are shown in the Table.

	University students (n = 100)	Gynaecology clinic attendees (n = 104)	Medical workers (n = 128)	Pregnant women (n = 107)
Mean age (range)	20.6 (16–24)	25.8 (16–44)	26.3 (16–45)	25.9 (18–37)
CT	12.0	6.7	4.7	4.7
NG	2.0	5.8	1.6	0.0

Half of *N. gonorrhoeae*-positive females were also infected with *C. trachomatis*. In univariate analysis, STDs were significantly associated with age under 25 years (p = 0.02), age at first sexual intercourse less than 18 years (p = 0.02), alcohol or drug intoxication during first intercourse (p = 0.003), migration from rural regions (p = 0.001), lower educational attainment (p = 0.037), and a higher number of lifetime sexual partners (p = 0.01). Marital status, family income, previous STDs, and condom use did not differ between infected and non-infected women. Among reported symptoms, vaginal discharge (p = 0.02) and intensive menstrual bleeding (p = 0.05) were more frequent in infected women.
Conclusion: The prevalence of *C. trachomatis* and *N. gonorrhoeae* infections in young females in Novosibirsk is similar to that in the USA. As in other populations, risky sexual behaviour has a significant effect on the risk of bacterial STDs.

P2155 *Chlamydia* and gonorrhoea in high-risk areas in Havana, Cuba: a point prevalence study

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Objectives: No studies on the prevalence of *Chlamydia* and gonorrhoea among the general population have been carried out in Cuba. We studied the prevalence of *Chlamydia* and gonorrhoea in a young, high-risk, population for contracting sexually transmitted diseases in Havana and describe associated risk factors for their transmission.
Methods: A random, point prevalence study was performed in a high risk population for contracting sexual transmitted diseases in urban areas of Havana, Cuba. 375 healthy young adults, 294 females and 81 males, with an age ranging from 14 to 35 years old gave their consent to participate. Participants older than 35 years, younger than 14, without previous sexual intercourse and those who were under current broad spectrum antibiotics or in the previous 4 weeks were excluded. A cervical smear in women and an urethral smear in men were used for *Chlamydia* and gonorrhoea testing (ProbeTec, BD, France).
Results: 5.6% (21/375) of the participants were positive for *Chlamydia*, 71.4% (15/21) were female and 28.6% male (6/15). Adolescent females at school age were identified as the highest risk group. Having a steady sexual partner for the last 6 months was not protective for infection (OR:1.86, p = 0.131), but more than 1 sexual partner in the last five year doubled the risk (OR:2.02; p = 0.147). Some well known complications of *Chlamydia* infection were already present among the infected group. Only 0.5% (2/375) participants tested positive for gonorrhoea.
Conclusions: *Chlamydia* infection prevalence in our study group appeared to be high enough to justify early diagnosis and treatment although it has the lowest prevalence in the Caribbean region. Focussing screening to teens older than 15 years at school would probably provide the most efficacious prevention approach in this scenario.

P2156 Practice of *Chlamydia trachomatis* testing; using laboratory surveillance and STI clinic data from 2002–2006

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There is limited knowledge about the testing practice of *Chlamydia trachomatis* (Ct). Considerable debate exists on the preferred testing policy.
The current study assesses practice of Ct testing and trends in Ct positivity rate.
Preliminary laboratory surveillance (LS) data of one laboratory and data from our STI clinic in The Netherlands (2002–2006) was used.
LS shows that between 2002 and 2006 the number of test requests for Ct has doubled. The main healthcare providers who request genital Ct testing are STI clinic (share of 27%), general practitioners (GP: 30%) and gynaecologists (Gyn: 37%). At most (92%) consultations only one anatomical site, mainly genital, from a person was tested. Anal Ct-tests were only requested by STI clinic. GP tested a higher share of women (75%) and persons were older (median age 28 years) than compared to STI clinic (62% women, median age 25 years). Median age of women tested by Gyn was 32 years.
The proportion of positive genital Ct diagnoses (positivity rate) increased slightly in men (9% in 2002 to 10% in 2006) and women (5% to 6%). This translated in increases in incidence, calculated using population data on the coverage area of the laboratory, from 33 to 96 per 100.000 for men and from 84 to 197 for women.
Although all providers requested more tests over time, the positivity rate increased only for attendees of the STI clinic. Notably, increases in men were not only caused by homosexual (4.4% in 2003 to 6.1% in 2006) but also by heterosexual men (5.3% to 8.4%).
Overall, genital Ct was diagnosed in 7.1% of men and 9.8% of women attending STI clinic, while for GP, this was 13.2% and 6.8%. For women tested by Gyn, the proportion was 3.1%. For all providers, the highest

positivity rate was observed among those 25 years of age or younger. Anal Ct was diagnosed in 5.7% of men and 8.2% of women.
Several healthcare providers increasingly test for Ct. Positivity rates increase as well, but only at the STI clinic where Ct testing is standard, and not at GP who opportunistically screen risk groups.
A possible explanation for the high positivity rate in men tested by GP is that GP seem to adopt a stricter test policy (only on indication) for men than for women. A negative consequence of this may be that an unknown number of Ct cases will be missed. Considering the relatively high proportion of positive anal Ct cases seen at our STI clinic, other healthcare providers should more often include this test in their test-package.

P2157 Prevalence and determinants of *Chlamydia trachomatis* infection among young women (14–24 years) in Turin, Italy

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Objectives: To estimate the prevalence of *Chlamydia trachomatis* infection in young women (14–24 years) from the general population and to determine risk factors associated with infection.
Methods: The study population consisted of sexually active young (⩾14 years old) women attending a public gynaecology clinic in the city of Turin (northern Italy) for a first visit between January 2002 and December 2005. *Chlamydia* was detected with the Ligase Chain Reaction test performed on an endocervical sample.
Results: A total of 1,849 young women were included in the study. The mean age was 21.6±2.1 years; 76.7% were Italian; 66.4% had two or more lifetime partners; 70.0% were single; 57.6% had a high school diploma. *Chlamydia* was detected in 6.2% of the study population. Among 407 asymptomatic women, the prevalence was 2.7%, whereas among the remaining 1,440 was 7.2%. Among 431 women originating from outside Italy, the prevalence was 9.3% (12.0% among 225 women from Eastern Europe, 7.3% among 124 women from Africa), and 5.3% among Italian women.
Chlamydia prevalence was 8.1% in the group aged 14–21 years and 4.8% in the group aged 22–24 years ($p < 0.005$). At the multivariate analysis, the positivity for *Chlamydia* was significantly associated with originating from outside of Italy (OR 2.62), high number of lifetime sexual partners (2–3 vs. 1 partner; OR 2.85), and having had more than one partner in the previous six months (OR 2.30).
Conclusion: The prevalence found in this study was higher compared to that reported in a previous Italian study conducted between 1997 and 2000 in a population of women of same age in the same geographic area (prevalence: 3.5%) (Latino et al., Sex Transm Inf, 2002). Even when disaggregating by presence of symptoms or by age group, the prevalence observed in our study was higher. This finding can be attributed to: a) higher proportion of foreign women in our study (23.3%) compared to the study of Latino et al. (9.6%); b) a true increase in incidence of *Chlamydia* infection after year 2000. The high prevalence observed among foreign women is probably associated with the high frequency of *Chlamydia* in their countries of origin. The risk factors for *Chlamydia* infection found in our study suggest that testing should be primarily offered to women younger than 22 years, even if asymptomatic, and also to foreign women and women with multiple partners.

P2158 Molecular evidence of *Chlamydia trachomatis* in a female patient with ocular lymphoma and chronic hepatitis B

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Objectives: Ocular adnexal non-Hodgkin lymphoma (OANHL), constitute a heterogeneous group of neoplasms that have been associated to a high prevalence of *Chlamydia psittaci* infection in tumour tissues and peripheral blood mononuclear cells (PBMCs) without evidence of other *Chlamydia* spp. or other microrganisms.
Methods: We carried out cell cultures and PCR methods to investigate *Chlamydia* spp. in tumour tissue and PBMC specimens collected from a patient with OANHL. The patient was a 53-years-old woman suffering from chronic B hepatitis, who was referred in September 2007 to the Section of Ophthalmology of University of Ferrara because of right ocular lymphoplasmacytic lymphoma (immunocitoma) of conjunctiva with microscopic evidence of typical salmon masses. In 2001, the patient had developed an analogous tumour in the left eye treated with radiotherapy. Common routine microbiological and serological tests were all negative for recent infections. Quantification of hepatitis B viral load showed highly detectable DNA viraemia. Because of the possible link between chlamydial infections and malignant lymphomas, the patient was referred to the Section of Infectious Diseases of our University to investigate Chlamydiae and other pathogens. DNA extraction from PBMC and lymphoma tissue samples was carried out following a standard phenol/chloroform extraction technique after proteinase K digestion. PCR with primer sets amplifying Chlamydiae gene 16s RNA and MOMP was assayed on DNA from fresh specimens, supernatant and cells culture (Hep-2).
Results: A PCR positive product (527 bp) corresponding to *C. trachomatis* MOMP was found in cultured tissues and PBMC specimens only. After sequencing, this fragment did show a strict homology with *C. trachomatis* (BLAST). No *C. psittaci* nor *C. pneumoniae* DNA sequences were detected. The patient was treated 4 weeks with doxycycline 100 mg, twice a day and PBMC chlamydial DNA did result negative after the conclusion of antibiotic treatment. A significant reduction of the previously described ocular lesions was observed.
Conclusions: This is the first study which demonstrates the presence of *C. trachomatis* DNA in PBMCs of a patient with ocular lymphoma. Like other chlamydiae, this pathogen causes persistent infection which may favour the development of OAL. The combination of culture with PCR has shown to improve the sensitivity of PCR thus suggesting its potential use to detect *C. trachomatis* and to monitor the efficacy of therapy.

P2159 Seroepidemiology of sexually transmitted infections in East and South Georgia, 1998–2002

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Objectives: Retrospective 5 year data analysis of patients seeking HIV, Syphilis, Chlamydia, and Gonorrhoea testing at public health clinics in suburban East (EG) and rural South (SG) Georgia from 1998–2002.
Methods: Using an ELISA and a confirmatory Western blot, 40094 individuals were HIV tested; 20199 (50%) lived in EG, 22718 (56%) were female, 15748 (39%) were Caucasian, 22127 (55%) were African American, 1132 (7%) identified themselves as men who have sex with men (MSM). 8663 (22%) were <20, 15080 (38%) between 20–29, 8696 between 30–39, and 7654 (19%) >40. From 1998–2002, 9142, 16984, and 766 cases of Gonorrhoea, Chlamydia, and Syphilis were reported respectively.
Results: Starting in 2002, HIV infection rate decreased in EG (1.1% in 1998, 0.5% in 2002; $p = 0.006$), but had remained stable in SG (0.8%). In EG, HIV infection was associated with gender (male, $p < 0.001$), race/ethnicity (African American, $p < 0.01$), age >30 ($p < 0.01$), sexual orientation (MSM, $p < 0.01$), and having a “sex partner at risk” ($p < 0.01$). In SG, HIV infection was associated with race/ethnicity (African American, $p < 0.01$), age >40 ($p < 0.01$), sexual orientation (MSM, $p < 0.01$), and having a “sex partner at risk” ($p < 0.01$), but not with gender. From 1998–2002, HIV seroconversion rates remained stable in SG (0.4%), but had ranged from 0.4–1.1% in EG. Only 50% of those testing HIV negative received post-test counseling. Gonorrhoea cases were higher in women (55% in EG, 58% in SG), African American (83% in EG, 67% in SG), and those <24 (68% in EG and SG). Chlamydia cases were higher in women (82% in EG, 87% in SG), African American (74% in EG, 51% in SG), and those <19 (41% in EG, 45% in SG). Syphilis cases were higher in men (54% in both EG and SG), African American (90% in EG, 80% in SG), and those >30 (58% in both EG and SG). In 2000 EG was composed of 57% Caucasian, and 52% female, while SG was composed of 73% Caucasian, and 50% female.

Conclusion: From 1998–2002, HIV infection rates was stable in South Georgia while it had decreased in East Georgia. Syphilis, Chlamydia, and Gonorrhoea cases remained stable in both areas. With recent reports of an increase in HIV cases in parts of Europe, and with the age of onset of sexual activities continuing to drop, education and prevention programmes need to continue to target teenagers, females, ethnic and sexual minorities. The importance of post-test counseling needs also be stressed, particularly to those who had tested HIV negative.

P2160 Quality of life in patients with recurrent urinary tract infections associated with sexually transmitted diseases – A healthcare problem

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Objectives: Probably 60% of women with sexual dysfunction and 61% of women with dyspareunia have recurrent urinary tract infection.
Methods: 120 patients with recurrent urinary tract infections (UTI) in age of 16–40 years old were examined from 2003–2007. Polymerase chain reaction (PCR), culture methods and serological tests were used for detection of sexually transmitted diseases, urinary culture for bacteriological tests of urine. Questionnaire "Pelvic Pain and Urgency/Frequency Patient Symptom Score" by L. Parson was used for quality of life tests.
Results: *E. coli* was detected in 55.1% (43) cases. Presence of agents of STD were found in 84.2% (101): *Ureaplasma urealyticum* in 34.5%(35) of patients, *Chlamydia trachomatis* in 20% (20) of patients, Herpes simplex I, II type in 25.2% (26) patients, in other 20% (20) cases it was mixed-infection. Pollakiuria up to 11–14 times per day in latent period had 31.6% (38) of patients, 96% (115) of women had dyspareunia, and because of pain and urinary tract symptoms during or after intercourse 92% (110) of patients avoid sexual life. Mild and extensive pain associated with bladder, pelvis, vagina, perineum, urethra during intercourse which leads to social and sexual disadaptation of women had 86% (103) of patients. Urgency and frequency after sexual intercourse had 88% (105) of patients, but extensive symptoms had only 28% (34)of patients. Symptom score less than 10 was in 8% (10), 10–14 in 28% (34), 15–19 – 36% (43) and more than 20 – 28% (34) of patients.
Conclusion: Recurrent urinary tract infections associated with STD in patents in young reproductive age lead to social and sexual disadaptation of women and decrease their quality of life.

P2161 Bacterial vaginosis and associated microflora in women of reproductive age

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Objective: The purpose of this study was to determine the prevalence of bacterial vaginosis (BV) and BV vaginosis associated microflora in women of reproductive age.
Methods: This study was conducted in a period of 6 months (January 1–June 30, 2007) and we evaluated a total of 2638 both vaginal and cervical swabs. They were collected from all women, aged 16–49 years, with and without symptoms of BV, who visited the Gynaecology Clinic. BV was diagnosed microscopically by Nugent's score and was defined as a modified Nugent score of 6 and above. Leukocytes detection in the Gram-stained slides was noted, too. Detection of aerobic and anaerobic bacteria and fungi was performed by standard microbiological methods. The swabs were plated on ship blood-, Thayer Martin-, Gardnerella- and Sabouraud-agar and when necessary, we used VITEK system (bioMérieux, France) to make a confirmation of the isolated anaerobes.
Results: Normal vaginal flora was microscopically detected in 2311 (83.8%). The prevalence of BV, Nugent's score above 6, was 10.2% (269 swabs), consistent with isolation of *Gardnerella vaginalis* in 243 (9.2%) cases. The rest 26 cases of BV represented isolation of other anaerobes (predominately *Bacteroides*). BV was most frequent (33%) in the younger age group (16–26 years). Furthermore, there were a total of 158 (6%) cases with leukocytes and Nugent's score 4–6. Out of those in 25 (15.8%) and 10 cases, anaerobic Gram-negative rods (*Bacteroides* or *Prevotella*) and anaerobic Gram-positive cocci (*Peptococcus* and *Peptostreptococcus*), were isolated respectively. In the evaluation period, Mobiluncus has not been detected. Other bacteria isolated from the total of the samples include approximately 10% streptococci (*Streptococcus agalactiae* and *Enterococcus*), 3% *Staphylococcus aureus* and 10% coliformes (predominately *E. coli* and *Klebsiella*), but not associated with any particular microflora. *Candida* spp. was found in 498 (22.0%) of all swabs.
Conclusion: BV, a syndrome characterised by a change of vaginal flora from predominant *Lactobacillus* to partial or total replacement with anaerobes has a higher prevalence in the younger age group. The isolation of *Gardnerella vaginalis* and other anaerobes is strongly associated with BV diagnosis by Nugent's score. Leukocyte detection is useful tool for microbiology diagnosis in women with vaginal discharge.

P2162 Bacterial vaginosis and vaginal leukorrhoea: an association with concomitant vaginal and cervical infections

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Objectives: Bacterial vaginosis (BV) is a vaginal condition characterised by the absence or scarcity of white blood cells (WBCs) in the vaginal discharge. We aimed to evaluate whether an increased vaginal WBCs in women with BV is associated with fungal, bacterial, or protozoan vaginal and cervical infections.
Methods: Our study was carried out over a 3-years period (9/2004–8/2007) and included 135 new nonpregnant premenopausal patients who attended the outpatient gynaecology dpt of our general hospital. All these women were diagnosed as having BV based on the Nugent scoring system. Vaginal leukorrhoea was defined as more than 10 WBCs per field X 400. Swabs were taken from posterior vaginal vault and the endocervical canal. Culture media included BAP, CHOC with IsoVitaleX, MTM, Sabouraud and MAC agar. *Trichomonas vaginalis* and *Candida* spp. detection was achieved by direct wet mount examination and testing for *Chlamydia trachomatis* was performed using chromatographic immunoassay.
Results: Of the 135 women enrolled in the study, 91 (67%) were symptomatic, while 44 (33%) were asymptomatic. The mean age was 26 years (range 17–48) and most of these women were less than 30 years old (98, 73%). Elevated vaginal WBC count was found in 19 (14%) women and the majority of them (15/19, 79%) were symptomatic. The prevalence of *Candida* spp.-*C. trachomatis*-*T. vaginalis*-*N. gonorrhoeae* in women with both BV and leukorrhoea was 21% (4/19), 37% (7/19), 21% (4/19), and 0% (0/19) respectively, whereas, in women without leukorrhoea the prevalence rates were 3.4% (5/116), 6.9% (8/116), 0% (0/116), and 0% (0/116) respectively. Leukorrhoea was statistically associated with *Candida* spp. ($\chi^2 = 7.35$, $P < 0.01$; OR 5.9, 95% CI 1.43 to 24.2), *C. trachomatis* ($\chi^2 = 14.8$, $P < 0.001$; OR 7.87, 95% CI 2.4 to 25.2), and *T. vaginalis* ($\chi^2 = 25.1$, $P < 0.001$). Women with both BV and leukorrhoea were significantly more likely to be co-infected ($\chi^2 = 45.5$, $P < 0.001$; RR = 7.04, 95% CI 4 to 12.2).
Conclusions: In the studied population, we found that among nonpregnant women with BV, vaginal leukorrhoea is strongly associated with co-infection with *Candida* spp.–*C. trachomatis*–*T. vaginalis* and these women were 7.04 times more likely to be co-infected than women without leukorrhoea. The prevalence of *C. trachomatis* in women with both BV and leukorrhoea (37%) is high and further tests should be performed to promote early detection and treatment.

P2163 Frequency of occurrence of *Mycoplasma genitalium* in women with unexplained (idiopathic) infertility

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Objectives: In the last 20 years, infertility has become a growing social and economic problem, especially in highly-developed countries. There

exist many defined and diagnosable causes of infertility of partners but idiopathic infertility, in the case of which it is impossible to identify the real reason of infertility using standard diagnostic methods, still stirs much controversy. *Mycoplasma genitalium* is a microorganism which may cause NGU, oligosymptomatic genital infections, disorders in the structure and mobility of sperm, and at the same time is impossible to detect in routine diagnostic tests. The objective of this study was to estimate the frequency of occurrence of *Mycoplasma genitalium* in the reproductive organs of women with infertility in comparison with a group of healthy patients who have given birth to at least one child. An attempt was also made to determine the correlation between the presence of the above species in the genital tract and fertility impairment.

Material and Methods: The study covered 51 patients with primary infertility, including 24 women with idiopathic infertility and 23 women with proven fertility. Cervical swabs and smear from the peritoneal cavity (pouch of Douglas) performed during laparoscopy. Presence of the genetic material of *Mycoplasma genitalium* in the material analysed using PCR.

Results: As a result of the analysis performed, *Mycoplasma genitalium* were found in the cervical channel of 19.6% of all infertile patients and in 4.4% of fertile patients. Additionally, the pathogen was discovered in the cervical canal of 29% patients with unexplained infertility, which in comparison with the group of fertile women was a statistically significant difference. In the abdominal cavity, *Mycoplasma genitalium* was found in 5.8% patients from the infertile group (in 8.4% patients with idiopathic infertility), while it was not detected at all in the laparoscopy material obtained from the studied fertile patients.

Conclusion: A significantly more frequent occurrence of *Mycoplasma genitalium* in the genital tracts of women with idiopathic infertility was found (both in the cervical canal and in the abdominal cavity) compared with the control group. The results obtained may suggest that *Mycoplasma genitalium* is a species having an impact on impaired fertility in women.

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P2164 A comparison of predictors for high-grade CIN in the triage of women with abnormal smears

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Objectives: It is well established that certain types of the human papillomavirus (HPV) are the primary risk factor for cervical cancer. Studies have shown that HPV DNA testing is substantially more sensitive than cytology for the detection of high grade cervical lesions, but lacks specificity. If HPV testing is to be used in cervical screening, improving the specificity is highly desirable. In this study we compared sensitivity, specificity and positive predictive value (PPV) of several adjunctive tests for the detection of high-grade disease (CIN2+ and CIN3+).

Methods: 954 eligible women aged 18 to 72 referred for colposcopy following one or more abnormal cervical smears participated in the study after having given written informed consent. Repeat cytology and up to six DNA or mRNA tests were carried out on a liquid PreservCyt® sample using commercially available kits or prototypes: Hybrid Capture II (Digene), Amplicor (Roche), HPV-Proofer (Norchip), APTIMA HPV assay (Gen-Probe), Linear Array (Roche), Clinical-Arrays (Genomica). p16INK4a immunocytochemistry was also performed. Sensitivity, specificity and PPV were based on the worst histology at baseline.

Results: 700 women (73.4%) had an abnormal repeat smear result (borderline or worse) including 541 (56.7%) with a mild dyskaryosis smear result or worse. Overall, 279 women (29.2%) had high grade disease (CIN2+) on worst histology, of which 173 (18.1%) had a CIN3+ result. Repeat cytology with borderline or worse considered positive had a sensitivity of 97.5%, specificity of 36.6% and PPV of 38.9% for CIN2+. For CIN3+ lesions, repeat cytology had a sensitivity of 98.3%, specificity of 32.1% and PPV of 24.3%. The performance of the different HPV tests and p16INK4a expression is ongoing and will be presented at the meeting.

Conclusions: Five of the tests (Amplicor, HCll, Linear Array, repeat cytology, APTIMA) had sensitivity >95%. Of these five tests, APTIMA shows the best specificity. HPV-Proofer was less sensitive than the other tests but much more specific.

In vitro susceptibility of *Candida* spp.

P2165 In vitro susceptibility of *Candida* species isolated from blood to common antifungal agents in a Spanish tertiary medical centre

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Objectives: To evaluate the susceptibilities of *Candida* blood isolates to common antifungal agents in the Hospital de Cruces, a Spanish tertiary medical centre.

Methods: A total of 206 blood isolates of different species of *Candida* were collected in the clinical routine in the period January 2004–October 2007. The yeasts were identified by conventional mycological methods. In vitro susceptibilities to 5-fluorocytosine (5FC), amphotericin B (AMB), fluconazole (FLC), itraconazole (ITC), ketoconazole (KTC), and voriconazole (VRC) were determined using the Etest method. MICs were recorded after 24 h of incubation at 37 ºC.

Results: The most frequently isolated species was *Candida parapsilosis* (87 isolates, 42.2%) followed by *Candida albicans* (79 isolates, 38.3%). Other non-*C. albicans* species, such as *Candida tropicalis* (17 isolates, 8.3%), *Candida glabrata* (10 isolates, 4.9%), *Candida guilliermondii* (7 isolates, 3.4%), or *Candida krusei* (4 isolates, 1.9%), were recovered in minor frequency. MICs at which 90% of the clinical isolates were inhibited were 0.5 μg/L for 5FC, 1 μg/L for AMB, 4 μg/L for FLC, 0.38 μg/L for ITC, 0.094 μg/L for KTC and 0.125 μg/L for VRC. AMB, VRC and KTC were very active in vitro against all blood isolates, even those that were resistant to ITC or FLC. A total of 23 isolates (11.2%) showed decreased susceptibility to ITC (MIC > 0.25 μg/L) and 10 isolates (4.9%) showed decreased susceptibility to FCZ (MIC > 8 μg/L). FLC resistance (⩾32 μg/L) was limited to 4 *C. krusei* isolates and to one isolate each of *C. glabrata* and *C. guilliermondii*. ITC resistance (⩾2 μg/L) was limited to 3 *C. glabrata* isolates, 2 *C. guilliermondii*, 2 *C. tropicalis* and 1 *C. krusei*.

Conclusions: Scarce antifungal resistances were found in *Candida* blood isolates from our institution. FLC resistance was limited to 4 *C. krusei* isolates and to one isolate each of *C. glabrata* and *C. guilliermondii*.

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P2166 Trends in susceptibility to fluconazole of yeasts isolated from patients in Saint Petersburg, Russia, during a 3-year period, 2004–2007

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Objectives: To determine prevalence of resistance to fluconazole in clinical yeast isolates in Saint Petersburg during the period from October 1, 2004 to September 30, 2007.

Methods: Evaluation of susceptibility to fluconazole was performed by disk-diffusion method according to CLSI M44-A protocol.

Results: The total of 1600 yeast isolates from various clinical materials (genital, oral, blood, urine, sputum, cerebrospinal fluid, bile, wound and other) were tested for susceptibility to fluconazole (511 strains were isolated during the period from October 1, 2004 to September 30, 2005; 586 strains – from October 1, 2005 to September 30, 2006 and 503 strains – from October 1, 2006 to September 30, 2007).

Candida albicans was most common – 1126 strains (70.3%), *Candida*-non-*albicans* – 414 (25.9%), *Cryptococcus neoformans* – 26 (1.6%), other yeasts – 34 (2.1%).
The number of resistant yeast strains during the whole period of investigation was 103 (6.4%), but prevalence of resistance to fluconazole ranged from 6.1% (1st year period) to 5.7% (2nd year period) and 7.6% (3rd year period). The incidence of susceptible-dose-dependent isolates was 4.3%, ranging from 2.9% to 4.9% and 5.8% during three successive years of observation. Most of the isolates (89.3%) were susceptible to fluconazole.
Prevalence of resistance to fluconazole in *C. albicans* isolates was 0.53% and ranged from1.0% to 0.0% and 0.6% during three years of observation.
Conclusion: Prevalence of resistance to fluconazole in the yeasts isolated from patients in Saint Petersburg during the period of 2004–2007 is low, but the number of resistant and dose-dependent strains has increased.

P2167 In vitro susceptibility of *Candida* species to micafungin: a comparison of methods

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Objective: Clinical isolates of *Candida* species (n = 123) were taken from sterile and non-sterile sites to determine in vitro MIC's and minimum fungicidal concentrations (MFC) to the echinocandins micafungin and caspofungin. Differing methodologies for testing micafungin were employed for comparison.

Table 1. GM MIC's and MFC's for mica fungin are shown for various *Candida* species determined using various methods

Species	n	Method	GM MIC (mg/L)	MIC range (mg/L)	GM MFC (mg/L)	MFC range (mg/L)
C. albicans	32	EUCAST	0.016	⩽0.015–0.06	9.722	0.125–>8
		ODDS	0.016	⩽0.015–0.03	0.355	⩽0.015–>8
		CLSI (24 h)	0.016	⩽0.015–0.03	2.406	⩽0.015–>8
		CLSI (48 h)	0.019	⩽0.015–0.5	0.392	⩽0.015–>8
C. glabrata	16	EUCAST	0.017	⩽0.015–0.125	0.040	⩽0.015–>8
		ODDS	0.017	⩽0.015–0.06	0.028	⩽0.015–1
		CLSI (24 h)	0.016	⩽0.015–0.06	0.028	⩽0.015–4
		CLSI (48 h)	0.017	⩽0.015–0.25	0.048	⩽0.015–>8
C. parapsilosis	12	EUCAST	1.130	0.5–4	9.665	4–>8
		ODDS	0.542	0.125–1	4.219	1–>8
		CLSI (24 h)	0.722	0.5–1	6.128	2–>8
		CLSI (48 h)	1.130	0.5–2	4.537	1–>8
C. tropicalis	12	EUCAST	0.017	⩽0.015–0.03	6.307	0.06–>8
		ODDS	0.016	⩽0.015–0.03	0.068	⩽0.015–0.25
		CLSI (24 h)	0.020	⩽0.015–0.5	1.577	0.03–>8
		CLSI (48 h)	0.024	⩽0.015–1	0.160	0.03–>8
C. krusei	11	EUCAST	0.108	0.06–0.25	0.354	0.25–1
		ODDS	0.108	0.06–0.25	0.149	0.125–0.25
		CLSI (24 h)	0.108	0.06–0.125	0.250	0.25
		CLSI (48 h)	0.124	0.06–0.25	0.210	0.125–0.25
C. guilliermondii	10	EUCAST	0.235	0.06–1	7.246	1–>8
		ODDS	0.248	0.06–0.5	10.767	1–>8
		CLSI (24 h)	0.472	0.125–1	16.000	>8
		CLSI (48 h)	0.500	0.25–0.5	16.000	>8
C. kefyr	10	EUCAST	0.043	⩽0.015–0.06	0.092	0.06–0.25
		ODDS	0.034	⩽0.015–0.06	0.080	0.06–0.25
		CLSI (24 h)	0.037	0.03–0.125	0.060	0.06
		CLSI (48 h)	0.040	0.03–0.06	0.080	0.03–0.25
C. lusitaniae	10	EUCAST	0.060	0.03–0.125	4.391	0.06–>8
		ODDS	0.039	⩽0.015–0.06	0.549	0.03–>8
		CLSI (24 h)	0.050	0.03–0.06	0.606	0.03–>8
		CLSI (48 h)	0.060	0.03–⩾8	0.606	0.03–>8
C. inconspicua	3	EUCAST	0.030	⩽0.015–0.125	–	–
		ODDS	0.026	⩽0.015–0.06	–	–
		CLSI (24 h)	0.030	⩽0.015–0.125	–	–
		CLSI (48 h)	0.035	⩽0.015–0.125	–	–

Methods: Four methods were employed to determine MIC and MFC values to micafungin: EUCAST, Odds (Odds et. al., 2004, JCM 42:3475), CLSI M27-A2 (24 h and 48 h incubation). Parameters for EUCAST, Odds and CLSI methods respectively were as follows: inocula: 1×10^5, 1×10^3 and 1×10^3 CFU/ml; media: 2% glucose, no additional glucose and 2% glucose; incubation period: 24, 24 and 48 h; endpoint calculation: 50, 50 and 80%. Caspofungin was tested using the Odds method and 24 h MFC's. Data analysis was performed substituting 16 for >8 mg/L. Drug dilution stability at –80ºC was assessed monthly using the EUCAST QC panel. For each species and method GM MIC and MFC values and ranges (mg/L) were calculated. MFC's (99% kill on 0.1 ml) for the CLSI method MFC's were recorded for both time points.
Results: Micafungin drug dilutions were stable for 6 months at –80ºC. All species exhibited low MIC values to micafungin however inter-species variation was observed. Where the GM MIC was relatively higher, e.g. *C. guilliermondii* and *C. parapsilosis*, the differing methods produced notable intra-species variation in MIC values. Reproducibility data (20%) was excellent. For *C. parapsilosis* MIC's to micafungin varied from 0.125–1.0, and caspofungin from 0.5–2 (Odds). MFC's for micafungin produced markedly different results dependent on the method utilised; the MFC value frequently ranged from <0.015 to >8 mg/L for a single isolate. A significant range of results for MFC's was noted for all species.
Conclusion: The differing methods did not produce markedly different MIC values for the echinocandin micafungin, with most isolates exhibiting low MIC's to the drug, typically lower than caspofungin. In contrast Micafungin and caspofungin MFC values varied significantly dependent on the method employed; this correlated with the inoculum level.

P2168 In vitro activity of micafungin combined with amphotericin B or fluconazole against *Candida parapsilosis*

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Objective: The aim of this study was to evaluate the in vitro activities of the combinations micafungin (MFG, Astellas, Japan) + amphotericin B (AMB, Sigma, USA) and MFG + fluconazole (FLC, Pfizer, Spain), against 30 blood isolates of *Candida parapsilosis*.
Methods: The minimum inhibitory concentrations (MIC) were determined according to Clinical and Laboratory Standards Institute guidelines for yeasts, and defined as the lowest drug concentrations that produced a 50% inhibition of visible fungal growth after 24 h of incubation. Drug interactions were assessed by the checkerboard microdilution method. Antifungal agents were placed in rows or columns of trays with MFG concentrations ranging from 0.06 μg/mL to 32 μg/mL, AMB concentrations ranging from 0.06 μg/mL to 4 μg/mL, and FLC concentrations ranging from 0.03 μg/mL to 2 μg/mL. The fractional inhibitory concentration index (FICI) was used to classify drug interaction as follows: FICI ⩽ 0.5, synergistic; 0.5 < FICI ⩽ 4, indifferent; and FICI > 4, antagonistic. *Candida krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019 were used as quality control strains.
Results: Overall, MFG MICs ranged from 2 μg/mL to 16 μg/mL. The MICs at which 50% and 90% of all the isolates tested were inhibited (MIC50 and MIC90, respectively) were 8 μg/mL and 16 μg/mL and the MIC geometric mean (GM) 7.64 μg/mL. AMB MICs ranged from 0.25 μg/mL to 1 μg/mL. The MIC50 and MIC90 were 0.25 μg/mL and 1 μg/mL, respectively, and the GM, 0.40 μg/mL. FLC MICs ranged from 0.25 μg/mL to 1 μg/mL. The MIC50 and MIC90 were 0.5 μg/mL and 5 μg/mL, respectively, and the GM, 0.41 μg/mL.
The highest percentage of synergy was obtained combining MFG + AMB (22.2%) and the lowest combining MFG + FLC (6.7%). The remaining combinatory results were indifferent.
Conclusion: The highest number of synergistic interactions against *C. parapsilosis* was found for the combination MFG + AMB but only against 22.2% of isolates.
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P2169 Influence of anidulafungin on different *Candida* species in a continuous flow culture system

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Objectives: We investigated the antifungal effect of anidulafungin (ANID) on biofilms and MIC values of different *Candida* species in a miniature flow cell system.
Methods: Strains of *C. albicans* (Ca), *C. glabrata* (Cg), *C. krusei* (Ck), *C. parapsilosis* (Cp) and *C. lusitaniae* (Cl) from blood culture isolates were grown in flow cells (0.16 mL). Peptone-yeast extract (0.5%/0.2%) + 50 mM glucose +/– ANID (8 mg/L) was supplied at very low flow rates (1.3 mL/h). Biomass production, glucose metabolism, pH and planktonic CFUs were measured. MIC testing was performed by Etest® on RPMI or Mueller-Hinton methylene blue agar. Morphology of adherent fungal cells was assessed microscopically after staining with lactophenol cotton blue.
Results: For Ca (3 isolates, 5 experiments) we found a strong decrease of biomass production, elevated concentrations of glucose and an increase in pH after continuous input of ANID. MICs of baseline strains were 0.003–0.004 mg/L. The same MIC values were found on day 2, 4 and 5. After 5 d sometimes, the biofilm did not show any growth. In one experiment, an increase of MIC to 0.75 mg/L was to be seen, but there was no development of resistance (no growth of biofilm on day 7). In 5 experiments with Cg (4 strains) glucose metabolism stopped within 24 h and no biomass remained after 3–4 d. MIC 0.012–0.016 mg/L. Ck biofilm (1 strain, 1 experiment) was most susceptible to ANID (MIC 0.004 mg/L) equally for baseline strain and biofilm. Cp (4 strains, 8 experiments) showed different degrees of susceptibility. In some experiments, we saw small inhibition zones (MIC 4–8 mg/L). In others, we found no inhibition and no production of biofilm under the influence of ANID. Cl (1 strain, 1 experiment) was found to be good susceptible to ANID. The produced biofilm stopped after ANID exposure (MIC 0.023 mg/L).
Conclusion: Under continuous flow conditions, the strains of Ca, Cg and Ck were most susceptible to ANID as described for static conditions. Cl was also very susceptible. Cp showed different degrees of susceptibility.

P2170 In vitro activity of anidulafungin combined with amphotericin B or fluconazole against *Candida parapsilosis*

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Objective: The aim of this study was to evaluate the in vitro activities of the combinations anidulafungin (AFG, Pfizer, Spain) + amphotericin B (AMB, Sigma, USA) and AFG + fluconazole (FLC, Pfizer), against 30 blood isolates of *Candida parapsilosis*.
Methods: The minimum inhibitory concentrations (MIC) were determined according to Clinical and Laboratory Standards Institute guidelines for yeasts, and defined as the lowest drug concentrations that produced a 50% inhibition of visible fungal growth after 24 h of incubation. Drug interactions were assessed by the checkerboard microdilution method. Antifungal agents were placed in rows or columns of trays with AFG concentrations ranging from 0.015 μg/mL to 8 μg/mL, AMB concentrations ranging from 0.06 μg/mL to 4 μg/mL, and FLC concentrations ranging from 0.03 μg/mL to 2 μg/mL. The fractional inhibitory concentration index (FICI) was used to classify drug interaction as follows: FICI $\leqslant$0.5, synergistic; $0.5 < \text{FICI} \leqslant 4$, indifferent; and FICI > 4, antagonistic. *Candida krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019 were used as quality control strains.
Results: Overall, AFG MICs ranged from 0.5 μg/mL to 4 μg/mL. The MICs at which 50% and 90% of all the isolates tested were inhibited (MIC50 and MIC90, respectively) were 2 μg/mL and 4 μg/mL and the MIC geometric mean (GM) 1.66 μg/mL. AMB MICs ranged from 0.25 μg/mL to 1 μg/mL. The MIC50 and MIC90 were 0.25 μg/mL and 1 μg/mL, respectively, and the GM, 0.40 μg/mL. FLC MICs ranged from 0.25 μg/mL to 1 μg/mL. The MIC50 and MIC90 were 0.5 μg/mL and 5 μg/mL, respectively, and the GM, 0.41 μg/mL.
The highest percentage of synergy was obtained combining AFG + AMB (33.3%) and the lowest combining AFG + FLC (6.7%). The remaining combinatory results were indifferent.
Conclusions: The highest number of synergistic interactions against *C. parapsilosis* was found for the combination AFG + AMB but only against one third of the isolates tested.
Funding: Projects PI061895 from Fondo de Investigación Sanitaria (Ministerio de Sanidad y Consumo of Spain) and IT-222–07 from Departamento de Educación, Universidades e Investigación (Gobierno Vasco).

P2171 In vitro susceptibility testing of fungal isolates from immunosuppressed, organ-transplanted patients for anidulafungin and azoles

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Objectives: Organ transplant recipients (OTR) are predisposed to mucocutaneous *Candida* infections. The aim of our study was to compare the minimum inhibitory concentration (MIC) of the newly introduced drug anidulafungin, an echinocandin, and already marked azoles (flu-, itra-, vori-, posaconazole) in strains obtained from this particular risk group in comparison to healthy controls.
Methods: From 400 OTR and 405 sex- and age-matched controls, two body sites were sampled for fungal culture (oral and genital mucosa/glans penis). Susceptibility testing was carried out in parallel for the azoles using the E-test (AB Biodisk, Sona Sweden). Results were read after 24 hours (MIC 80). For anidulafungin MIC (90) was obtained by broth microdilution method according to CLSI standard and determined visually and by spectophotometry after 48 h.
Results: From 529 isolates (273 from OTR, 256 from controls) susceptibility tests were carried out (418 oral mucosa, 111 genital area/mucosa). The number of *Candida* non-*albicans* species was significantly increased in OTR in the late post-transplantation period (>18 months) compared to the early period and the controls. For fluconazole, 2.2% (n = 1) of *C. glabrata* were resistant (MIC $\geqslant$ 64ug/ml), for itraconazole, 82.6% (n = 38) of *C. glabrata*, 66.7% (n = 2) of *C. krusei* (MIC $\geqslant$ 1ug/ml); all strains were susceptible for voriconazole (MIC $\leqslant$ 1ug/ml). For posaconazole and anidulafungin no reference values for break points exist. For posaconazole, 10.9% (n = 5) of *C. glabrata* showed a MIC (80) $\geqslant$4ug/ml (all other strains range 0.002–0.38 ug/ml). *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*, *C. lusitaniae*, *C. norvegensis* were most susceptible for anidulafungin (MIC (90), 0.03 to 0.25 ug/ml); *C. parapsilosis* and *C. dubliniensis* were the least susceptible (MIC (90), 0.25 to 16 ug/ml). *C. guilliermondii* was neither isolated from OTR nor from controls. With regard to in vitro activity there was no difference between strains from OTR and controls, no shift towards decreased susceptibility of the *Candida* strains obtained in the late post-transplantation period or with the type of organ transplanted.
Conclusion: With the exception of *C. glabrata*, *Candida* spp. obtained from OTR exhibit very good in vitro activity towards azoles. With regard to anidulafungin all *Candida* strains from these patients including *C. glabrata* showed an excellent in vitro activity with the exception of *C. parapsilosis* and *C. dubliniensis* showing higher MICs than other *Candida* spp.

P2172 Anidulafungin exhibits potent activity against multi-echinocandin multi-azole cross-resistant *Candida parapsilosis* isolates

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Objectives: Echinocandins (ECs) are typically less active against certain *Candida* species, especially *C. parapsilosis* (Cp) and *C. guilliermondii* (Cg), but are believed to have similar activities. However, recent case reports suggest that differences in in vitro activity do exist.
Materials: We evaluated and compared the in vitro activity of the 3 ECs [anidulafungin (afgn), caspofungin (cfgn), and micafungin (mfgn)] and two azoles (fluconazole and voriconazole) against 76 *C. parapsilosis* (Cp) and 13 *C. albicans* (Ca) isolates (obtained from patients, healthcare workers, and the hospital environment) using the Clinical Laboratory Standards Institute M27-A2 methodology. Effects of the ECs on the cell morphologies of cfgn/mfgn-susceptible and -non susceptible isolates were assessed using scanning electron microscopy (SEM). Expression of efflux pumps CDR1/CDR2 and MDR1 were analysed by Northern blotting. Additionally, analysis of the FKS-1 gene to determine point mutations associated with reduced EC susceptibility was also evaluated.
Results: We found that Cp isolates obtained from burn unit patients were more susceptible to afgn (MIC 2 μg/ml), than to cfgn or mfgn (MICs 8 μg/ml). Furthermore, it was not uncommon to observe CP that showed multi-EC resistance to Mfgn-Cfgn, while remaining susceptible to afgn. However, Cp isolates obtained from healthcare workers or environmental sources were remained susceptible to all of the antifungals examined. SEM revealed that incubation of cfgn-susceptible and -non-susceptible Cp strains with afgn led to cell damage and eventual death. Low concentrations of afgn (2 μg/mL), but much higher concentration of cfgn (16 μg/mL), were needed to induce morphological changes in a cfgn-non susceptible strains of Cp. MDR1 expression was upregulated, while FKS1 mutations were not detected in sequential isolates that exhibited multi-EC cross-resistance.
Conclusions: This study demonstrates the emergence of EC-cross resistance in one institution. The in vitro studies demonstrate that the activity of all ECs is not the same. In fact, in these Cp isolates, the antifungal activity of afgn was more effective and quite different then the in vitro activity of cfgn and mfgn against certain Cp isolates. The results also suggest that the cross-resistance detected in these isolates appears to be mediated by multiple factors. The clinical impact of these multi-EC isolates are yet to be determined.

P2173 Effect of protein-binding on the in vitro efficacy of echinocandins against *Candida* species

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Objective: The echinocandin (EC) class of antifungals demonstrate a high degree of protein-binding (>95%) for all 3 ECs. Several studies have suggested that this high degree of binding may decrease the efficacy of the ECs against fungi in vivo. The aim of this study was to investigate the differential effect of serum on the in vitro activity of the ECs against different *Candida* spp. and compare them to the activity of azoles and amphotericin B.
Methodology: 160 *Candida* isolates were evaluated and included 58 *C. albicans* (Ca), 48 *C. glabrata* (Cg), 14 *C. parapsilosis* (Cp), 10 *C. tropicalis* (Ct), 10 *C. krusei* (Ckr), 10 *C. lusitaniae* (Cl), and 10 *C. kefyr* (Cke). MICs and time kill assays were used to evaluate the in vitro efficacy of anidulafungin (Afgn), caspofungin (Cfgn), micafungin (Mfgn), amphotericin B (AmB), fluconazole (Flz), and voriconazole (Vcz). MICs were performed using CLSI M27-A2 methodology in either RPMI or RPMI/50% human serum to determine and compare the differential in vitro activities. Time kill assays (TKA) in RPMI and in RPMI/50% serum were performed fo all six antifungals against one strain each of the different *Candida* spp. All assays were conducted in duplicate.
Results: In general, 50% serum significantly decreased the activity of all 3 ECs in vitro, from as low as 2-fold flucuations to as much as a 64-fold increases in MIC. Cfgn had the narrowest alterations in MICs. The greatest differences were seen among Cke and Ckr with a 64-fold change in EC activity with and without serum. In addition, to complement the MIC data, TKA also demonstrated similar decreases in activity with all 3 ECs, especially Cfgn against Ckr. In contrast, the in vitro activity of AmB, Vcz, and Flz did not change when the MICs were performed in 50% serum or in RPMI.
Conclusion: In general, the data supports the fact that serum consistently and dramatically decreases the in vitro activity of all 3 ECs in vitro, and this effect was demonstrated in all 3 ECs. In contrast, the decrease in activity in serum was not observed with AmB, Flz, or Vcz. For the ECs, the significance of this decrease in in vitro activity in serum is not fully understood and the clinical impact of this alteration in efficacy has yet to be determined.

P2174 Activity of echinocandins against *Candida albicans* strains with decreased susceptibility to fluconazole isolated from APECED patients

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Objectives: Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) is an autosomal recessive disease exceptionally common in Finland. Chronic mucocutaneous candidosis (CMC) is the most common component of APECED and most patients suffer from it since early childhood. Most patients receive repeated treatment and maintenance courses of azole antifungals, principally ketoconazole and fluconazole, throughout their lives. This has resulted in mycological and clinical resistance. The aim of this study was to determine the activity of the echinocandins caspofungin, anidulafungin and micafungin against 43 *Candida albicans* isolates from Finnish APECED patients treated and followed in Helsinki University Central Hospital.
Methods: *C. albicans* isolates from 10 APECED patients reported to have decreased fluconazole sensitivity (n = 18, MIC range: 16–32 mg/L) and from 17 APECED patients reported to be sensitive to fluconazole (n = 25, MIC range: 0.120–8 mg/L) were tested for their sensitivity to caspofungin, anidulafungin and micafungin. The *C. albicans* strains had been isolated during the years 1994–2004. A broth dilution technique as detailed in the CLSI document M23-A2 was used.
Results: All *C. albicans* isolates were sensitive to the three echinocandins tested. The mean MIC of all isolates was 0.048 mg/L (range 0.007–0.600) for caspofungin, 0.037 mg/L (range 0.007–0.120) for anidulafungin and 0.020 g/L (range 0.007–0.060) for micafungin. Decreased fluconazole susceptibility did not affect the susceptibility to the echinocandins.
Conclusions: All three echinocandins tested were found to be highly active against *C. albicans* strains from APECED patients. No cross-resistance between triazoles and echinocandins could be seen since strains with decreased fluconazole susceptibility were found to be susceptible. Micafungin was found to be the most potent echinocandin against *C. albicans* strains with decreased fluconazole susceptibility.

P2175 A global evaluation of voriconazole activity against recent clinical isolates of *Candida*

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Objectives: Approved by the FDA in 2002, voriconazole (VOR) has been widely used for prevention and treatment of invasive fungal infection. As a result, prospective surveillance for emergence of in vitro resistance to VOR is indicated.
Methods: From January 2004-December 2006, we collected 5615 invasive (blood or sterile site) clinical isolates of *Candida* from 78 centres in 30 countries worldwide (by region: 1011 from Asia-Pacific [AP], 1829 Europe [EU], 1118 Latin America [LA], 1657 North America

[NA]). In vitro susceptibility to VOR was performed at a central lab using the CLSI M27-A2 method and CLSI breakpoints to classify organisms as susceptible (S), susceptible dose-dependent (SDD), or resistant (R). We present results overall, by species, by country, and region. In addition, we compare in vitro activity of this recent collection with that of 5866 isolates collected during global surveillance from 1997–2001, prior to the availability or use of VOR.

Results: Species distribution was 54% *C. albicans*, 14% *C. glabrata*, 14% *C. parapsilosis*, 11% *C. tropicalis*, and 3% *C. krusei*. VOR was very active overall: MIC50/MIC90, 0.007/0.25 μg/mL; 98% S. Of the 96 isolates not S to VOR (n = 66 R, 30 SDD), 87 (91%) were *C. glabrata*, primarily from centres in NA and EU. Finland had the highest rate of VOR resistance (10% R, 8% SDD, 82% S among 73 total isolates). In all other countries, ⩾97% of *Candida* were S to VOR. No temporal trends in species or MIC distribution were noted over the 3 years of surveillance. In comparison to 5866 *Candida* isolates collected during global surveillance from 1997–2001, there were no changes in mean MIC or MIC distribution (mean MIC = 0.13, MIC90, 0.25 μg/mL for both collections).

Conclusion: VOR is highly active in vitro against a global collection of recent invasive *Candida* isolates, and there is no evidence for emerging resistance or "MIC creep" compared with isolates collected prior to VOR availability. VOR SDD and VOR R isolates were primarily *C. glabrata* isolates from centres in NA and EU.

P2176 Comparison between Isavuconazole and other azoles against characterised clinical isolates and yeast model systems

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Objectives: Isavuconazole (ISA) is a novel azole with a broad activity range and is evaluated in clinical trials. In order to compare ISA against other azoles utilised in the therapy of fungal diseases (Fluco-, FLC; Vori-, VRC, Itra-, ITR; Posaconazole, POS), *Saccharomyces cerevisiae* (Sc) was used as a host for the expression of *Candida albicans* (Ca) genes involved in azole resistance (ABC transporters CDR1, CDR2 and Major Facilitator (MF) MDR1; ERG11 alleles with mutations) and 16 pairs of Ca matched clinical isolates with known azole resistance mechanisms were investigated. Testing of azole susceptibility among these different yeast isolates allows quantitative comparisons between the above-mentioned azoles.

Methods: Sc isolates expressing CDR1, CDR2, MDR1 and ERG11 alleles were described elsewhere (AAC 39: 1995). Sequential Ca clinical isolates with increasing MICs to several azoles were obtained from the University Hospital Centre and their azole resistance mechanisms described elsewhere (EC 3: 1994; EC 6:2007). The collection of clinical Ca isolates (38) is representative for most of the existing azole resistance mechanisms and their combinations. Susceptibility testing was performed according to CLSI standards using RPMI for clinical isolates and YNB for Sc.

Results: When transporters were expressed in Sc, results showed that FLC, VRC, ITR, POS and ISA were substrates of CDR1 and CDR2, since their expression resulted in relative MIC increase to all azoles tested as compared to control. On the opposite, the expression of MDR1 did not increase POS, ITR and ISA MIC, suggesting that these azoles were poor substrates for MDR1. When mutated ERG11 alleles were expressed, relative increases of azole MIC (from 4- to 32-fold) were observed for FLC, VOR and ISA when at least 2 mutations were present in the same ERG11 allele, whereas no MIC increase were measured when ITR and POS were tested. These results suggest that ERG11 mutations have limited effect on ITR and POS activity. Upon MIC testing of clinical Ca isolates with azoles, the MIC90 for FLC, VRC, ITR, POS and ISA were 128, 2, 1, 0.5 and 2 μg/ml, respectively, and showed that ISA had comparable activities than ITR, POS and VOR.

Conclusions: Resistance mechanisms of clinical isolates have differentiated impact on azole MIC depending on the azole structure. The main resistance mechanisms in yeasts involving ABC transporters and ERG11 decrease the activity of ISA, while MDR1 has limited effect.

P2177 In vitro post antibiotic effect of isavuconazole, voriconazole and amphotericin B against a strain of *C. albicans* with concentration-dependent pharmacokinetics in a murine model and susceptibility of strains for murine kidneys post infection

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Objective: Isavuconazole (ISA) is a water soluble triazole suitable for IV and oral dosing in Phase III trials. Understanding the pharmacodynamics (PD) of antimicrobials is a essential in defining the treatment schedules for optimum outcomes. Using a strain of *C. albicans* used to determine the PD of ISA the post-antifungal-effect (PAE) of ISA, voriconazole (VCZ) and amphotericin B (AmB) were determined in vitro. Also the susceptibility of this *C. albicans* strain was determined from kidneys of mice following treatment with low or high dose ISA.

Method: PAEs were determined in RPMI broth + 2% glucose buffered with MOPS. Log phase growth cultures (10^6 CFU/mL) were exposed to, solvent, ISA or VCZ at 0.1, 0.5, 1, 2, 5, 10, 40 and 100 × MIC; AmB cultures were exposed at 2 × MIC. Following 3 hours of exposure all drugs were removed by serial washing prior to dilution and transfer to fresh pre-warmed media. Cultures were incubated on an orbital shaking and viable CFUs determined hourly for 8 h then at 11 and 21 h. The log10 count was plotted against time and PAE defined as the difference is the time required for the count in solvent and drug exposed cultures and to increase 1×log10 above the count following drug removal. Mice were immunocompromised with cyclophosphamide then infected with the same strain of *Candida* then treated 5 h post infection with vehicle 6 or 24 mg/kg of ISA. 24 h post treatment mice were culled and kidneys cultured onto Sabouraud agar plus ISA (containing 0.25–2.5 × MIC). Recovered fungi had MICs tested by CLSI M27A2.

Results: The in vitro PAE of ISA was absent at ⩽MIC for ISA but was 2–2.5 hours for all doses ⩾2 × MIC. The PAE was much shorter <1 hour for all doses of VCZ. As expected a much longer PAE of 11 hours was seen with AmB. The burden following infection of 2.7×10^5 for vehicle, 1.7×10^5 for 6 mg/kg and 9.5×10^3 for 24 mg/kg was recovered from drug free plates. ~75% of this burden was recovered from ISA containing plates following prolonged incubation. Colonies recovered from ISA containing plates had identical MICs to the input cultures indicating no generation of resistance.

Conclusions: ISA demonstrated very significant PAE at drug concentrations ⩾2× MIC. Plasma and tissue ISA levels of >100× *Candida* MICs are easily attainable in humans and animal models so PAE might have a significant effect on the therapeutic response and PD. *Candida* recovered from organs post treatment had MICs unaffected by drug exposure.

P2178 Antifungal susceptibilities of *Candida* bloodstream isolates from two different Turkish hospitals

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Background: *Candida* spp. are among the leading aetiologic agents of bloodstream infections in hospitalised patients. Antifungal susceptibilities of these isolates are very important for the management of patients with invasive candidiasis.

Objectives: This study was performed to investigate the in vitro antifungal susceptibility patterns of *Candida* bloodstream isolates from two different hospitals located in the same city.

Methods: A total number of 139 non duplicate *Candida* bloodstream isolates (49 *C. albicans*, 24 *C. parapsilosis*, 11 *C. tropicalis*, eight *C. glabrata*, three *C. kefyr*, three *C. utilis*, two *C. guilliermondii*, one *C. famata*, one *C. krusei* and one *C. lusitaniae* from Dokuz Eylul (DEU) hospital; 12 *C. albicans*, 16 *C. parapsilosis*, three *C. famata*, three *C. pelliculosa* and two *C. guilliermondii* from Dr Behcet Uz Children's Hospital (BU)) cultured within one year period were tested against amphotericin B, fluconazole and voriconazole using CLSI M27-A2 microdilution method. The identification of the isolates were done

according to germ tube test, morphology on corn meal tween 80 agar and CHROMagar *Candida* and API 20C AUX system.

Results: The MIC50 and MIC90 values detected for *C. albicans*, *C. parapsilosis*, *C. tropicalis* and *C. glabrata* strains are shown in the table. All isolates except one *C. lusitaniae* were susceptible to amphotericin B. One *C. krusei*, one *C. parapsilosis*, three *C. tropicalis* isolates were resistant to fluconazole while the rest of the strains were susceptible. For voriconazole one *C. albicans*, two *C. tropicalis* and one *C. utilis* were detected to be resistant and one *C. albicans* was found as dose dependent susceptible.

Candida species	MIC for DEU isolates (μg/ml)						MIC for BU isolates (μg/ml)					
	AMB		FLU		VORI		AMB		FLU		VORI	
	50	90	50	90	50	90	50	90	50	90	50	90
C. albicans	0.125	0.25	0.25	0.50	0.015	0.06	0.125	0.25	0.25	0.50	0.015	0.03
C. parapsilosis	0.125	0.25	0.50	2.0	0.015	0.06	0.125	0.25	0.5	1.0	0.015	0.015
C. tropicalis	0.25	0.50	4	64	0.06	4						
C. glabrata	0.25	0.25	4	8	0.06	0.5						

Conclusion: According to our results it can be concluded that amphotericin B, fluconazole and voriconazole are detected to be active against our *Candida* bloodstream isolates and they remain to be appropriate choices for the treatment of patients with *Candida* bloodstream infections.

P2179 Identification rate and susceptiblity of *Candida* isolates from blood cultures

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Objective: The incidence of invasive candidiasis has increased over the past 2 decades and it is an important cause of complications and death in hospitalised patients. Due to the life-threatening nature of these infections and increasing drug resistances, susceptibility testing of *Candida* spp. has crucial importance. During a 2-year period (2005–2007), *Candida* infections were analysed in two institutions, the 1208-bed University Hospital in Halle, Germany, and the 1240-bed University Hospital in Szeged, Hungary.

Methods: Results of 41.423 blood culture samples were reviewed. The samples had been analysed by BacT/ALERT® 3D (bioMérieux) and BACTEC™ (BD) automated systems in Halle and Szeged, respectively. The maximum incubation time was 10 days. All positive samples were microscopically examined by Gram stain. For the identification of *Candida* species, we used CHROMagar® *Candida* (MAST DIAGNOSTICA/BD, rice agar, Auxacolor 2 (Sanofi Diagnostics Pasteur), ID 32 C and the VITEK® 2 YST card (bioMérieux).

In vitro antifungal activities of amphotericin B, flucytosine, fluconazole, itraconazole, voriconazole, posaconazole, caspofungin and anidulafungin were investigated by Etest® (AB Biodisk).

Results: Of the examined blood culture samples (1–13 samples/patient, mean 3 samples/patient), 16.7% were positive for bacteria and/or fungi. The most prevalent isolates were aerobic bacteria, found in 91% of the positive cultures. *Candida* spp. were detected in 172 patients. They constituted the second most common isolates, with a mean isolation frequency of 6.3%. Anaerobic bacteria were found in 2.7% of the positive cultures.

Most specimens positive for *Candida* spp. were received from intensive care units, from patients with cancer, organ transplants, burns and surgical infections. The most prevalent species was *C. albicans* (55.8% of isolates). From each of the 172 patients positive for *Candida* spp., one representative isolate was referred to further investigation. All tested isolates were sensitive to amphotericin B, voriconazole and posaconazole. Only one isolate of *C. albicans* and 6 isolates of *C. parapsilosis* displayed decreased susceptibilities to both caspofungin and anidulafungin (MIC > 1 μg/mL).

Conclusion: The new antifungals posaconazole and anidulafungin have excellent in vitro activities against invasive isolates of *Candida* spp.

P2180 Resistance to antifungal drugs of yeasts isolated from genital specimens

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Objectives: Identification of yeasts in genital specimens and testing their sensitivity to various antifungal drugs, in order to appreciate the rate of resistance to the most used of them. The study was realised during one year, September 2006-September 2007. All the specimens were collected from women with clinical symptomatology of infection present

Methods: We used CANDIFAST kit (International Microbio-France), which allows the identification of the main human pathogenic yeasts as well as testing their sensitivity to antifungal agents: polyene Nystatine and azoles Flucytosine, Econazole, Fluconazole, Miconazole. Due to the fact that the strains were isolated from vaginal samples, we tested them also to Ketoconazole, by disk diffusion method

Results: We analysed 160 specimens of vaginal secretions that had positive cultures on Sabouraud gelose. From 160 isolated strains, 142 were identified as *Candida albicans*, 12 strains identified as *Saccharomyces* sp., 4 strains as *Candida glabrata* and 2 strains as *Candida krusei*. *Candida glabrata* strains were resistant to Flucytosine, Miconazole, Fluconazole and Clotrimazole. *Candida krusei* were resistant to Nystatine, Flucytosine, Miconazole Fluconazole and Clotrimazole. *Saccharomyces* sp. did not presented resistance to the tested drugs. For *Candida albicans* strains the most important resistant rates were obtained for Ketoconazole (in 122 strains – 85.9%) and the lowest rate of resistance were for Nystatine and Econazole (in 12 strains – 8.6%).

Conclusions: *Candida albicans* remains the most frequent fungus isolated from genital infections. The sensitivity to antifungal drugs was good, in vitro, for Nystatine, Econazole, Clotrimazole Miconazole, Fluconazole and Flucytosine. We found important resistance in vitro to Ketoconazole. The difference between these two groups was significant.

P2181 Rapid determination of the antifungal susceptibility of *Candida* isolates using direct samples of blood from patients with fungaemia: the role of the Etest

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Objectives: Prompt and adequate antifungal treatment is crucial for good outcome in patients (pts) with fungaemia. Although antifungal susceptibility testing can help, standard procedures are laborious. Even with commercial systems such as Sensititre YeastOne® (SYO), results may take 48–72 h after diagnosis. We prospectively performed antifungal susceptibility testing by the E-Test (ET) on direct blood samples from 65 pts with fungaemia (88 strains) and compared it with SYO.

Methods: We included 86 different blood culture sets (BACTEC): *C. parapsilosis* (37), *C. albicans* (27), *C. tropicalis* (9), *C. glabrata* (8), *C. guilliermondii* (2), *C. glabrata* + *C. albicans* (2), and *T. mucoides* (1). We inoculated 10 drops from 1 bottle of a hemoculture set from each pt onto RPMI 1640 agar and placed ET strips of amphotericin B (AMB), fluconazole (FLU), voriconazole (VRC), posaconazole (POS), and caspofungin (CAS) on the agar. The MIC was read after 24 h of incubation. Breakpoints for FLU, VRC and POS (similar breakpoints than for VRC) followed CLSI recommendations. Strains with MICs >>1 μg/mL for CAS and AMB were considered resistant. Errors were very major (VM) when a strain was susceptible by ET and resistant by SYO, and major (M) when a strain was resistant by ET but susceptible by SYO.

Results: The % of strains classified as correct/VM/M for each agent was POS (100/0/0), FLU (98.8/0/1.2), VRC (98.8/1.2/0), AMB (58.1/38.4/3.5), and CAS (91.9/8.1/0). VM errors detected for CAS were only seen in *C. parapsilosis*. The 73 strains from purportedly FLU-susceptible species (*C. parapsilosis*, *C. albicans*, and *C. tropicalis*) were correctly classified by ET for the 3 azoles. Of the remaining 13 strains from species with low susceptibility to azoles (*C. glabrata*,

C. guilliermondii, and *T. mucoides*), 100% were correctly classified for POS and only 7.7% were M for FLU (*T. mucoides*) and VM for VRC (*C. glabrata*). The % of strains with MIC discrepancies of no more than ±2-fold dilutions between both methods were 76.7, 68.6, 70.9, 54.6, and 81.2 for POS, FLU, VRC, AMB, and CAS, respectively.
Conclusions: Overall, ET correctly classified >97% of strains for azoles, and 85% of species with low susceptibility to FLU. ET is rapid, easy, and can determine the antifungal susceptibility of azoles against isolates from pts with fungaemia. J. Guinea is contracted by the Fondo de Investigación Sanitaria (contract number CM05/00171). This study was financed by grants from CIBER RES CD06/06/0058.

P2182 Comparison of *Candida* susceptibility testing between CLSI broth micro-dilution and Vitek AST-YS01 card

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Objective: To compare yeast susceptibility testing between CLSI broth micro dilution method with the VITEK 2 using the AST-YS01card (bioMerieux, Baumes-Les Grotte, France).
Materials and Methods: 82 clinical isolates were tested, among which 21 *Candida albicans*, 23 *C. glabrata*, and *Candida* sp. other than *albicans* or *glabrata*. CLSI Broth micro dilution method (M27-A) was performed in the following way. Micro dilution plates containing 100 μl of the twofold serial dilutions of the antifungal drugs in standard RPMI 1640 medium were inoculated with 100 μl of yeast suspension containing between 1.0×10^3 and 5×10^3 CFU/ml. Plates were incubated at 35ºC, and MICs were determined after 24 and 48 h. Reference MICs corresponded to the lowest drug dilution that showed prominent growth inhibition (50% or more). The VITEK 2 ST-YS01 card was used according to the manufacturers guidelines. The AST-YS01 card contains the antifungal agents Amphotericin B, Fluconazole, Flucytosine and Voriconazole in four dilutions Each card also contains an growth control. The total incubation time is maximal 36 hours.
Results: A total of 82 clinical isolates of 12 different *Candida* species were tested. For *Candida albicans* all isolates had similar values for all antifungals. For other *Candida* species, all isolates had identical MIC's for amphothericin B. 11 (13.4%) isolates had different MIC levels for fluconazol and in six (7.3%) instances this lead to a different interpretation of susceptibility. Five of these 11 isolates were *Candida glabrata* but only in one case this resulted in a major interpretation error (R > S). For flucytosine 2 of 82 (2.4%) isolates had discrepant values (2.4%) leading to different interpretation For voriconazole for 12 (14.6%) isolates different values were found but only in one instance this lead to a different interpretation.
Conclusions: The VITEK 2 is a reliable method for determining the susceptibility of *C. albicans*. For other yeast species the results for amphothericin B, flucytosine and voriconazole appear to be reliable but for fluconazol and *C. glabrata* have to be interpreted with caution.

Viral and fungal emerging infectious diseases

P2183 Preliminary evaluation of a commercially available rapid immunochromatographic method for the detection of IgM antibody response to chikungunya and dengue viruses

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Objectives: Chikungunya virus (CHIKV) belongs to the family of arthropod-borne viruses and it's transmitted to humans by Aedes mosquitoes in tropical and sub-tropical areas. In August 2007, the first epidemic outbreak within the territory of the EU has started in the province of Ravenna in North-Eastern Italy. Up to today more than 200 laboratory confirmed cases have been reported and more than 1000 patients underwent laboratory investigation. The large diffusion of Aedes albopictus (the tiger mosquito) in Northern and Central Italy was the condition that allowed the epidemic diffusion of CHIKV. The laboratory diagnosis is performed by detecting the viral RNA in blood by RT-PCR within the first 6 days after onset and by serological evaluation immediately after this period.
Methods: The presence of an IgM specific response to CHIKV and Dengue was evaluated by two different methods: immunofluorescence (IIFT) test (Euroimmun) and a newly available immunochromatographic (IC) method (CTK OnSite Duo) that is designed to distinguish between IgM response to CHIKV and Dengue. The samples used were obtained, during the recent outbreak, from 67 different patients with clinical and epidemiological evidence of suspected acute CHIKV infection (high fever and multiple arthralgia) living in the area of Ravenna.
Results: 45 samples were detected negative for IgM against CHIKV and Dengue by using IIFT and 50 specimens were scored as negative by IC. No IgM reaction against Dengue were detected. The use of IIFT allowed to identify as positive for CHIKV 22 samples but only 17 were confirmed with the IC method. The relative sensitivity of IC (versus IIFT chosen as the reference method) is 90%. The relative specificity of IC is 100%.
Conclusion: The use of a quick and simple test, like the IC evaluated in this study, could be proposed as a "field" method to rapidly discriminate the true CHIKV infections during epidemic outbreaks. The availability of a rapid response is useful for the appropriate targeting of the environmental interventions devoted to the control of vector mosquitoes. A correct environmental intervention has been demonstrated as the most efficacious methods to stop the spread of CHIKV during the recent outbreak in Italy. The use of "field" tests is not sufficient to achieve a correct diagnosis of CHIKV infection and the use of more sensitive laboratory tests such as IIFT is mandatory to confirm the clinical suspect.

P2184 Early diagnosis of dengue infection using blood and non-blood specimens: a pilot study

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Objectives: Dengue infection is the most wide-spread mosquito-borne disease worldwide. Serologic diagnosis is often made retrospectively upon clinical recovery. Our group has demonstrated the value of late febrile and early postfebrile urine and oral specimens in dengue virologic and serologic diagnoses. In this study, we sought to determine clinical utility of reverse transcription-polymerase chain reaction (RT-PCR) using early febrile blood and non-blood specimens for virologic diagnosis of dengue infection.
Methods: Adults with acute fever of no more than 3 days and without obvious organ-specific symptoms during June 2006 to October 2007 entered the study. Saliva, buccal brush, urine, plasma, and peripheral blood mononuclear cells (PBMC) were collected and tested by dengue-specific RT-nested PCR with primers targeting conserved regions of the 3′ untranslated region of the virus. Where available, 3 consecutive specimens from febrile days 3, 4, and 5 were tested. Diagnosis of dengue infection was based on positive standard ELISA assay on paired serum/plasma specimens. Those with negative dengue ELISA tests served as a control group.

Specimens	Sensitivity	Specificity	PPV	NPV
Urine	6/12 cases (50%)	12/12 cases (100%)	6/6 cases (100%)	12/18 cases (66.7%)
Saliva	4/12 cases (33.3%)	11/12 cases (91.7%)	4/5 cases (80%)	11/19 cases (57.9%)
Buccal brush	4/12 cases (33.3%)	11/12 cases (91.7%)	4/5 cases (80%)	11/19 cases (57.9%)
Plasma	12/12 cases (100%)	11/12 cases (91.7%)	12/13 cases (92.3%)	11/11 cases (100%)
PBMC	12/12 cases (100%)	11/12 cases (91.7%)	12/13 cases (92.3%)	11/11 cases (100%)
Saliva + Buccal brush	6/12 cases (50%)	11/12 cases (91.7%)	6/7 cases (85.7%)	11/17 cases (64.7%)
All non-blood specimens	9/12 cases (75%)	11/12 cases (91.7%)	9/10 cases (90%)	11/14 cases (91.7%)

Results: Of over 30 enrolled patients, 24 were eligible for analysis. Secondary dengue infection was diagnosed in 12 patients, leaving the other 12 as negative controls. The virus was detected in urine in half of the patients. The results were the same using both saliva and the buccal brush. With all non-blood specimens combined, three-fourths of the patients were detected. Plasma and PBMC both provided perfect yields (Table).
Conclusion: This is the first study demonstrating utility of both blood and non-blood specimens for early dengue virologic diagnosis. Even though this is only a pilot study, the results are promising. We are further performing the study in more patients. A similar study in paediatric patients is also under way.

P2185 Serum TNF-alpha and interleukin-4 (IL-4) levels in patients with Crimean-Congo haemorrhagic fever

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Objectives: Crimean-Congo haemorrhagic fever (CCHF) is a zoonotic infection mainly transmitted by ticks. In Turkey, spordic and endemic CCHF cases have been reported.
The aim of this study is to compare serum TNF-alpha and interleukin-4 (IL-4) levels in patients diagnosed with CCHF with those in healthy controls.
Methods: Forty-two adult patients diagnosed with CCHF based upon clinical and laboratory findings and 40 healthy adult volunteeers were included in the study. The diagnosis of CCHF was made with IgM positivity or 4 fold increase in IgG titration and/or Real-time polymerase chain reaction (RT-PCR) positivity. In the sera of patient and control groups, TNF-alpha and IL-4 levels were measured according to recommendations of the manufacturer.
Serum TNF-alpha and IL-4 levels in patient and control groups were compared statistically by using Student's t test.
Results: Serum TNF-alpha and IL-4 levels were found to be significantly higher in patients with CCHF than those found in healthy controls (p:000, $p < 0.05$ significant). Results are shown in the Table.

Table. Serum TNF-α and IL-4 levels in patients with CCHF and healthy subjects

	Number	TNF-α (μg/ml)		IL-4 (μg/ml)	
		Median	Range (min–max)	Median	Range (min–max)
CCHF	42	15.01	6.98–70.86	1.07	0–5.6
Control	40	7.69	4.06–11.87	0.00	0.00–7.14

Conclusion: Tha fact that serum TNF-alpha, a proinflammatory cytokine, and IL-4, an anti-inflammatory cytokine, levels were found to be significantly higher in patients with CCHF than those in control group suggests that cellular immune response (Th1 and Th2) may play an important role in this disease. In order to determine the role of cytokines in the pathogenesis of CCHF, further studies with larger samples are required.

P2186 A survey of Crimean-Congo haemorrhagic fever virus in tick populations and wild-life animals in Turkey

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Objectives: CCHF epidemic which was recognised in 2002 is still expanding regarding both of the clinical cases and geographical distribution in Turkey. The role of the vector ticks and wild-life animals was not studied nationwide. In this study, we aimed to clarify these aspects of the CCHF in Turkey.
Methods: A total of 3236 tick samples from the domestic and wild animals, and ground were collected from epidemic and non-epidemic centres from all of geographic regions of Turkey. The study was conducted between April through October 2006 and 2007. Blood samples were collected from 89 hunted wild boars and hares from exclusively epidemic regions throughout the year. Collected ticks were pooled as 5–20 samples according to their species and collection sites and screened for the presence of CCHF virus. Viral RNA was extracted from ticks and animal blood samples using commercial blood or/ tissue RNA extraction kits. Following reverse transcription, a part of CCHF virus S segment were amplified with specific primer sets by nested PCR amplification. PCR products of the positive samples were sequenced using ABI 310.
Results: Three of wild-boar blood samples, 2 of wild hare blood samples, 13 pools of ticks collected from hunted animals and 6 pools of ticks collected from domestic animals were found as positive for CCHF virus. Positive pools were encountered in both endemic and non-endemic sampling sites. Viral RNA was detected in Hyalomma marginatum, Rhipicephalus bursa, Boophilus annulatus and *Dermacentor marginatus* pools. In this study we obtained some interesting results; One of the wild boars hunted in early spring harboured CCHF virus, and we found viral RNA in an unfed *D. marginatus* sample which has not been previously shown to be involved in CCHF epidemiology in Turkey. Sequenced isolates were similar to those encountered previously, namely Europe II clade.
Conclusions: Based on the results of this study, CCHF virus is widely distributed in Turkey, including epidemic and non-epidemic centres. Although the role of migratory birds can not be excluded; wild-life animals and their ticks may play a major role for the propagation and spreading of the virus between domestic and wild animals and human populations. The positiviy of wild boars in early spring, and a tick species, *D. marginatus*, which is active throughout the year suggests that CCHF virus may maintained in wild-life also during the winter season in which the major vector ticks is non-active.

P2187 2007 outbreak of viral meningitis in Romania: cases managed in a national institute of infectious diseases

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In 1996 in south-eastern part of Romania a meningitis epidemic due to West Nile virus affected 863 persons. Therefore, in June 2007 when a new meningitis epidemic occurred we decided to perform a prospective analysis of these cases. We made a descriptive study about the cases admitted in our Institute.
Objective: to analyse the cases of meningitis in order to identify a possible aetiological agent.
Methods: we excluded the cases of bacterial meningitis (tuberculosis included). We analysed 132 cases of meningitis and the case definition was: fever, meningian syndrome and CSF with more than 7 leukocytes/mm3.
Results: Mean age was 23.8 years (between 14 and 72 years), the predominance of male, equivalent distribution regarding rural versus urban provenience and water distribution were described. Most of cases were admitted in August (40.15%) and in July (28.7%). Clinical description: moderate cases, without encephalitis syndrome, with moderate meningian syndrome in 46% of cases. 56 patients associated sinusitis, 65.9% had moderate increased level of fibrinogen and 15.9% had more than 12000 leucocytes /mmc. CSF examination showed in 95.3% of cases – less than 500 cells/mm3. CSF protein was increased but the value was below 2.5g/l for all patients (below 1g/l for 81.8% of cases). For 23 patients we isolated ECHO 4 virus from CSF. All the patients received pathogenic and symptomatic medication and the outcome was favourable in all the cases. This epidemic was different than that due to West Nile, which associated encephalitis, affected old people and had unfavourable outcome in 20% of cases.
Conclusions: 2007 viral meningitis epidemic affected young people, male, and outcome were favourable in all the cases.

P2188 Haemorrhagic fever with renal syndrome and leptospirosis in Champagne-Ardenne: comparison of clinical, biological and epidemiological pictures

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Objectives: The haemorrhagic fever with renal syndrome (HFRS) caused by Puumala virus and the leptospirosis are two zoonoses found in Champagne-Ardenne. Their clinical, biological and epidemiological profiles are relatively similar. The aim of this study is to compare these profiles for an effective therapeutic direction.

Methods: A retrospective study led from 1995 to 2005 included 44 HFRS cases and 45 non-icteric leptospirosis hospitalised during the study period. HFRS diagnosis rests on the serology – presence of IgM or increase of IgG rate (44/44); and leptospirosis diagnosis rests on the Martin Petit serodiagnosis (38/45).

Results: The number of cases per year, the monthly distribution, the average age and the sex ratio do not show any significant differences between the two pathologies. Leptospirosis mainly concerns occupational hazards – farmers – and leisure activities like swimming; and HFRS concerns foresters, walks in forests and cleaning of attics. Lumbago ($p < 0.001$), abdominal pains ($p = 0.007$), visual trouble ($p = 0.003$), rise of blood pressure ($p < 0.001$) and pharyngites ($p = 0.01$) are more frequently found in HFRS. ASAT ($p = 0.006$), CPK ($p < 0.0001$) and CRP ($p < 0.0001$) are higher in leptospirosis, whereas creatinine ($p = 0.009$) is higher in HFRS.

Conclusions: Only visual trouble is really specific to the HFRS diagnosis, even though differences are observed between the two pathologies in the biological and clinical profiles.

Thus, the quick screening test of IgM by immuno-chromatography regarding HFRS is useful to the differential diagnosis allowing then an effective therapeutic direction.

P2189 Preliminary study on possible risk co-factors in the transmission route of HHV-8 with promoter blood sucking arthropods. Survey through questionnaires in two sub-Saharan African countries: Cameroon and Senegal

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Objectives: Human herpesvirus-8 (HHV-8) infection in Africa occurs mostly in childhood and through interfamilial contacts. This oncogenic virus maybe considered as an emergent pathogen due to its spreading in connection with the AIDS pandemic. Assuming as working hypothesis that blood sucking promoter arthropods are involved in the transmission of HHV-8, we observed the following possible risk-cofactors related to the virus infection route: i) the saliva of seropositive mothers used on children's skin to relieve itching, ii) the use of traditional medication based on crushed and often premasticated herbs, and so probably contaminated with saliva, iii) the local inflammatory reaction related to child's hypersensitivity response to the bite and iv) fever as systemic symptom.

Methods: We carried out survey with questionnaires in students (age 5–13) interviewed at schools in Cameroon and in Senegal.

Results: In Cameroon 200 questionnaires revealed the following frequencies: use of traditional methods (113/200) 56.5%; saliva (22/200) 11% and herbs (91/200) 45.5%, fever after bite (83/194) 42.8%, skin irritation and swelling, respectively (96/200) 48% and (135/200) 67.5%, lasting of skin boring: <3 days (136/185) 73.5%, 3–7 days (37/185) 20% and >7 days (12/185) 6.5%.The relation between traditional methods and fever was not statistically significant ($p < 0.055$) but the variable fever showed statistically significant direct relation with use of herbs ($p < 0.001$) and fever more frequent if the lasting of skin boring time is >3 days ($p < 0.001$). In Senegal 104 questionnaires revealed the following frequencies: traditional methods (23/89) 25.8%; saliva (15/89) 16.9%, herbs (8/86) 9.3%, fever (25/99) 25.3%, irritation (6/98) 6.1% and swelling (85/98) 86.7%, lasting of skin boring: <3 days (86/90) 95.6%, 3–7 days (1/90) 1.1% and >7 days (3/90) 3.3%. As in Cameroon the variable fever showed statistically significant relation with herbs ($p < 0.007$) and with skin boring lasting for >3 days ($p < 0.01$). Furthermore there was a statistically significant relation with skin reaction ($p < 0.013$).

Conclusion: Data show that swelling, fever when the skin boring is lasting >3 days and herbal-based medication, are possible risk-cofactors in the transmission route of HHV-8 with promoter arthropods. Further surveys will be directed to analyse the kind of blood sucking arthropods involved in the transmission and the effect of salivary gland proteins and of herbs on the reactivation of the HHV-8.

P2190 A natural focus of hantavirus infection in the Šumava region (Czech Republic)

M. Pejcoch, E. Pauchova, B. Kriz (Prague, Brno, CZ)

Objectives: To find whether the human cases in the Prachatice district (CR) are of hantavirus origin and if so, to identify the causative genospecies and map its circulation in the natural focus likely to be the reservoir of infection.

Methods: Sera from patients with suspected HRFS and occupationally exposed individuals were screened for anti-hantavirus antibodies by commercial ELISA kits from Progen Biotechnik. Terrestrial rodents were captured in the suspected areas. The specific PUU antigen was detected from homogenised lungs of these animals using the commercial kits Hantagnost from the Chumakov Institute of Poliomyelitis and Viral Encephalitides of the Russian Academy of Medical Sciences, Moscow.

Results: Hantavirus infections are emerging diseases as the causative agent was only recognised in 1978. More than 20 hantavirus genospecies have been identified to date. In Central Europe, three hantavirus genospecies, i.e. Dobrava-Belgrade (DOB), Puumala (PUU) and Tula (TUL), are known to circulate in natural foci. Their respective hosts are yellow-necked mouse (Apodemus flavicollis), bank vole (Myodes glareolus) and common vole (Microtus arvalis). In Eurasia, the major hantavirus disease is haemorrhagic fever with renal syndrome (HFRS). In Central Europe, the most serious infection is caused by DOB. TUL is generally non-pathogenic for humans. In the Czech Republic (CR), hantavirus infections have been reported sporadically. In the Czech Republic, the seroprevalence of hantaviroses in adults is about 0.8%. A local outbreak of hantavirus infection was observed in 2004 in the Šumava region, with some cases still persisting.

Eight patients admitted to the Prachatice district hospital with suspected HFRS and interstitial nephritis as the major symptom had primarily IgM antibodies against PUU. Two of the screened individuals showed seropositivity for IgG antibodies. The PUU antigen was detected from the lungs of a bank vole captured in the area where the forest workers had been working.

Conclusion: We detected a PUU natural focus in the Šumava region with hantavirus infection cases in humans. PUU was detected in its specific host Myodes glareolus from this biotope. The PUU focus is adjacent to another one situated on the other side of the border and reported previously by German authors. These findings need further study.

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P2191 Passive immunisation protects cynomolgus macaques against Puumala hantavirus challenge

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Objectives: Hantaviruses cause two severe and often fatal human diseases: haemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS). Presently, there is no effective prevention available for HFRS or HPS. Here, we studied the effect of passive immunisation on the course of infection in cynomolgus macaques challenged with wild-type Puumala hantavirus (PUUV-wt).

Methods: A pool of serum, drawn from previously PUUV-wt-infected monkeys, was used for immunisation, and a pool of serum from the

same monkeys that was obtained before infection was used as a control. Immunisations were administered three days before and 15 days after challenge with PUUV-wt. After challenge, monkeys were sampled once a week and analysed for markers of PUUV-infection.
Results: All three monkeys treated with non-immune serum became positive for PUUV-RNA in plasma and showed PUUV nucleocapsid-specific IgM-responses after challenge. In contrast, no PUUV-RNA or anti-PUUV specific IgM-response was detected in the three passively immunised monkeys. As seen in PUUV-infected humans, the control monkeys showed a marked decrease in the amount of platelets and increased levels of creatinine, IL-6, IL-10, and TNF after inoculation. In contrast, no marked changes in the amount of platelets were observed in the immunised monkeys and they did not show elevated levels of creatinine, IL-6, IL-10, or TNF after virus challenge.
Conclusion: The results show that passive immunisation in monkeys, using serum from previously hantavirus-infected individuals, can induce sterile protection and protect against pathogenesis. Convalescent-phase antibodies may represent a potential therapy that can induce immediate protection against HFRS and HPS.

P2192 Acute Chagas' disease in a European stem cell transplant recipient

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Objective: We report a recent case of acute transfusion-acquired Chagas disease in a bone-marrow transplant recipient in Spain.
Chagas disease is a major public health problem in endemic areas of South America. Untreated immigrants from endemic areas are a potential source of infection and spreading to nonendemic areas mainly via infected blood products used in transfusions.
Acute transfusional cases in immunosuppressed patients can be fatal unless early recognised and treated.
Methodology: Case report. A 33-year-old male patient received an allogeneic stem-cell transplantation (11/05/07) from a related donor because of bone-marrow aplasia diagnosed 2 months earlier. He received several blood transfusions (both red cells and platelets) before and after the procedure. On the 9th day after transplantation spiky fever developed, followed by blurred vision and palpebral edema, which quickly progressed to facial edema and erythema with palpebral purplish discolouration. All microbiological and radiological screening was negative. Fever persisted despite extensive empirical antibiotic and antifungal treatment. Erythema and edema became generalised and a skin biopsy was performed showing *Trypanosoma cruzi* amastigotes. Direct microscopy (×1000 magnification) of thick fresh blood showed motile trypomastigotes, also visible in Giemsa stained thin blood film (Fig. 1).

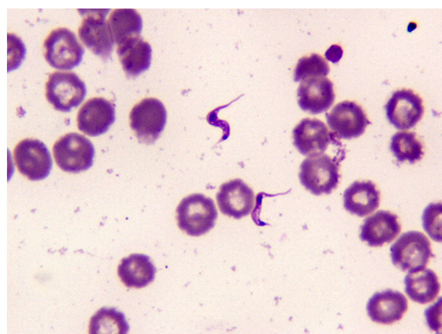

Figure 1.

PCR was positive for *Trypanosoma cruzi* in blood, bone-marrow, skin and urine. Chagas serology was negative. Neither the patient nor the donor had ever visited endemic Chagas areas.
Benznidazole, 300 mg qd, was started. Fever resolved in 7 days and visible parasitaemia disappeared in 10 days. PCR became negative in one month. Edema did gradually regressed. Treatment was completed with no major toxicity.
Tranfusional Chagas disease was then searched. Two months before stem cell transplantation, the patient had received platelets from an asymptomatic Bolivian donor, with positive Chagas serology.
Conclusions:
1. In the era of globalisation Chagas Disease has to be considered in the diagnosis of persistent fever in severely immunosuppressed patients.
2. Fever and facial edema and erythema were the most striking symptoms. Diagnosis is easy when the disease is focused and searched
3. Nonendemic areas must accelerate full implementation of a mandatory screening of blood donors from endemic areas, including organ donors and recipients. Immigrant pregnant women should also be screened to further prevent vertical transmission

P2193 Update on *Scedosporium/Pseudallescheria* infections

R. Horré, M. Lackner, S. de Hoog, J.P. Bouchara on behalf of the ECMM-ISHAM Working Group on Pseudallescheria/Scedosporium Infections

Objective: *Pseudallescheria boydii* and *Scedosporium prolificans* are truly emerging fungal pathogens. They cause local and/or disseminated infections in human and animals. In 2002, a "Working Group on *Pseudallescheria/Scedosporium* Infections" was established by the European Confederation Of Medical Mycology (ECMM) and accepted as worldwide working group by the International Society For Human And Animal Mycology (ISHAM) in 2006. The aim of this working group is to collect strains and data, to improve our knowledge in the pathogenic mechanisms of these fungi, and to develop new diagnostic methods and treatment options. A summary of the latest results of the members of this working group will be presented.
Methods: Studies have been performed about different human and fungal perspectives, such as: (1) clinical significance studies, (2) taxonomical research, (3) selective detection using different techniques (e.g. serology, PCR, culture, histopathology), (4) resistance studies, (5) treatment investigations, (6) virulence factors, (7) epidemiology, and (8) ecology.
Results: For better understanding of these fungi as human and animal pathogens results have been obtained with
- a recently developed selective culture media, which was used for the isolation from environmental, as well as, from clinical specimen
- taxonomic evaluation and reclassification
- resistance studies
- occurrence in artifical and native environment
- analyses of published case reports

Conclusion: Since the first meeting of the ECMM (-ISHAM) Working Group on *Pseudallescheria/Scedosporium* Infections (April 2004), lots of interesting results have been achieved and established by our worldwide network of members.
We welcome interested physicians to support our work by sending strains, participating in studies, or sending data. Further information is available on our website: http://www.scedosporium-ecmm.com.

P2194 Parasitaemia with *Plasmodium falciparum* in a patient after cardiac surgery and blood transfusion in a non-endemic country

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Objectives: Physicians should be aware of the possibility of post-transfusional malaria and blood smear should be included in the clinical work-up of patients with fever of unclear origin.
Patients and Methods: We describe the case of an Argentinean patient who developed fever 15 days after coronary artery bypass surgery (CABS).

Results: the patient required plasma, platelets and red cells transfusion (2, 10 and 2 units respectively) during surgery; 15 days later he complained of fever; physical examination revealed a mildly-ill-appearing man with a temperature of 39ºC but otherwise not remarkable findings. Laboratory data revealed mild pancytopenia. Blood and urine cultures were negative, hepatitis B, C and HIV viral load were negative as were serology for these viruses. A blood smear revealed *Plasmodium falciparum* gametocytes. The rapid malaria diagnostic test (NOW® ITC Malaria Test; Binax, INC., Portland, ME) was strongly positive detecting HRPII antigen (*P. falciparum* specific) and mild-positive for the common *Plasmodium* sp. antigen. Serology (IFAT) and PCR were also positive for *P. falciparum*. Treatment with quinine sulphate and doxycycline was begun; 2 months later the patient was asymptomatic and control blood smear were negative. Fourteen donors were associated with the transfusions but none had epidemiological background of travel to *P. falciparum* malaria areas; one donor refused to be tested; *Plasmodium* sp. serology was negative in 13 of them; PCR and chromatography were negative in 12 donors (these test could not be done in one of the donors). An investigation of all the patients who were operated at the same surgery-room and the same day was also negative for epidemiological malaria contact. Another patient received red-cells from the donor who refused to be tested but no other case of post-transfusion malaria was reported in the region.
Conclusions: The aetiology of malaria infection in the reported case remains unknown but might be linked to blood transfusion as there is no other epidemiological background although malaria due to the establishment of *Plasmodium* sp. in an indigenous or aircraft-travelling mosquito remains a possibility. Increases in international exchanges and migration from endemic areas enhance the possibility that blood donors might have been in contact with malaria parasites. Accurate histories of potential exposure to blood transfusions are necessary in the work-up of patients with fever.

Paediatric infections

P2195 Isolated renal hydatid cyst in a child: case report and review on renal hydatidosis

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Introduction: Hydatid disease is endemic in many parts of the world. Urinary tract involvement develops in only 2–4% of all cases, and isolated renal cysts are extremely rare. Renal hydatidosis in children is rare. There are no specific signs or symptoms that will reliably confirm the diagnosis of renal echinococcosis. A serological markers are not sensitive for the diagnosis of a hydatic cyst, it may be confused with a cyst in the organ involved. We wanted to emphasize the importance of keeping in mind the possibility of hydatid disease in cystic renal lesions, particularly in endemic regions.
Case report: A 13-year-old female was admitted to our clinic with a 3-month history of moderate preumblical pain. During the last 20 days, these symptoms had worsened. There was no history of recurrent urinary tract infection, calculi, or lower urinary tract symptoms. On physical examination, we found abdominal tenderness, and systemic examination was unremarkable. Urinalysis and results of laboratory studies were within normal range. A detailed history was obtained from the patient. She had been living in Zahedan city, and her family raised livestock. She had a history of contact with sheep and cattle. Ultrasound revealed a cortical cystic mass 9×8cm in size at the left kidney, but do not including multiple small cysts with internal echo. Computed tomography confirmed this lesion. It showed a unilocular cystic lesion without internal septa which originated from the left kidney and bulged superiorly to the kidney. Liver and other abdominal structures were all normal. Chest films were also normal.
The patient underwent surgical exploration of the left kidney with flank incision. After ruling out malignancy and the diagnosis of a renal hydatid cyst, the whole cyst layers were removed. During the postoperative period, albendazole treatment was started. Pathological examination confirmed the diagnosis of a renal hydatid cyst.
Conclusions: To conclude, this case emphasizes that hydatidosis should be considered in the differential diagnosis of any cystic lesion, especially in regions where it is endemic.
To avoid misdiagnosis, a careful examination of cystic lesions in kidneys should be carried out in endemic areas for the detection of hydatid cysts.

P2196 *Turicella otitidis* as a cause of middle ear infection: some clinical and bacteriological aspects

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Objectives: *Turicella otitidis* is a newly described non fermentative coryneform bacterium isolated almost exclusively from the ear exudates of cases of middle ear infection. The role played by this bacterium in the pathogenesis of acute and chronic otitis media is still a controversial issue.
Method: The clinical manifestations of all the cases of middle ear infections associated with *T. otitidis* were obtained by a retrospective analysis of the patients' medical charts. Microbiological analysis including identification and antimicrobial susceptibility testing was done at the Clinical Microbiology Laboratory of the Karolinska University Hospital, Stockholm, during the period from January 2002 to September 2005.
Results: We recovered *T. otitidis* in the ear swabs of 13 patients presenting with otitis media. The patients studied were 7 males and 6 females (mean age 7 years, median age 3 years). Five patients presented with bilateral otitis media (38%), and a perforated tympanic membrane was observed in 8 cases (62%). A more protracted course with frequent relapses and repeated antibiotic courses over a period of months or years were noticed in 3 patients (23%). Two patients (15%) suffered complications attributable to their middle ear infections namely mastoiditis in one patient and facial paralysis in another. In only two patients (15%) a second pathogen was identified namely *Moraxella catarrhalis* in one patient and *Pseudomonas aeruginosa* in another. Identification of the organism was primarily carried out by the API Coryne (bioMérieux, Marcy lÉtoile, France) and was further confirmed by the typical Gram-staining reaction and the colonial morphology. All the isolates were susceptible to penicillin by the Etest. Amoxicillin with or without clavulanic acid was the most widely used first line treatment.
Conclusion: *T. otitidis* is an easily identified and well defined microorganism that is often missed by most microbiology laboratories. Its clinical role in the pathogenesis of middle ear pathologies is still unresolved and further studies are required for this purpose.

P2197 Antibiotics for nasopharyngitis are associated with a lower risk of office-based physician visits for acute otitis media within 14 days for 3- to 6-year-old children

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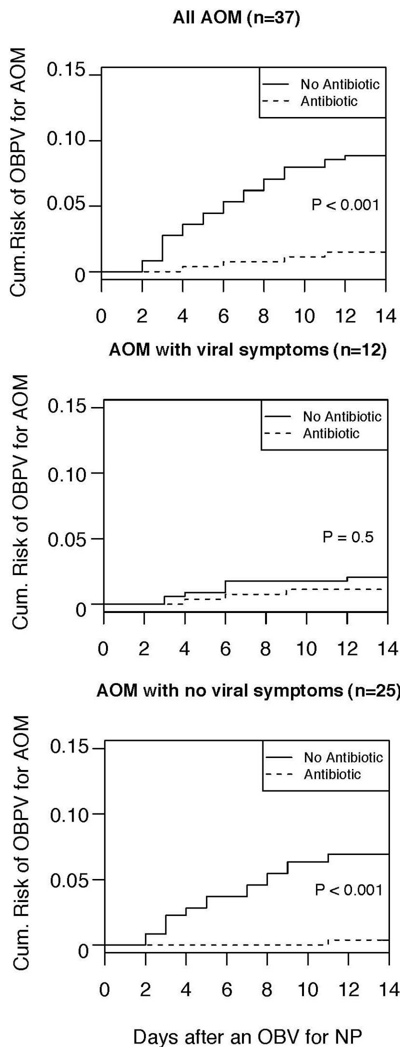
Objectives: This study was designed to analyse factors potentially influencing children's return visits to physicians for symptoms of acute otitis media (AOM) within 14 days after being diagnosed with nasopharyngitis (NP), and the impact of recent antibiotic use.
Design: A controlled population-based pharmacoepidemiological trial in 3- to 6-year-old children conducted from January to May 2000.
Setting: Three different geographical regions in France.
Participants: Among 2507 eligible children, 2456 could be analysed and 505 children had 634 office-based physician visits (OBPV) for NP symptoms.
Interventions: The statistical associations between antibiotics prescribed for NP and an OBPV for AOM within 14 days in a population-based study were analysed along with risk factors of AOM.
Main outcomes measure Clinical events and antibiotic use.
Results: During the 2 weeks following physician-diagnosed NP, antibiotic use, especially a β-lactam, significantly decreased the risk of OBPV for AOM in children (OR = 0.2, 95% CI=0.09 to 0.7; P = 0.002).

Conclusion: Antibiotics prescribed to children for NP seem to protect during the following 2 weeks against the risk of OBPV for AOM. It remains to be determined whether a subgroup at high risk of developing AOM after a viral infection exists and what might be the best strategy to adopt for NP in a national programme of optimal antibiotic use.



P2198 Evolution of nasopharyngeal bacterial populations during acute and convalescent phase of otitis media

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Objectives. The source of middle ear pathogens is nasopharynx. Normal microbiota of human nasopharynx is a complex ecosystem which consists of hundreds of different bacterial species. The role of pathogenic bacteria in acute otitis media (AOM) is well established, but the role of normal nasopharyngeal bacteria in the development and recovery of acute otitis media is still unclear. Further, both qualitative and quantitative data of the composition of the nasopharyngeal microbiota in general, is scarce.

The aim of this ongoing study is to follow the evolution of the bacterial populations of nasopharyngeal microbiota during acute otitis media and during the recovery process of otitis media.

Methods. The project is linked to a randomised, double-blind placebo-controlled trial of the antibiotic treatment of acute otitis media. The participating young children are closely followed for one year on regular visits. Nasopharyngeal specimens are taken on study days 1, 8, 15, 30, 60, 180, and 365. On day 1, patients with acute upper respiratory tract infection without AOM will serve as a control group. Both semi-quantitative culture-based methods in combination with accurate molecular identification as well as DNA-based culture independent methods are used for analysing nasopharyngeal microbiota.

Results. This far more than 1000 nasopharyngeal specimens have been collected and analysed of which more than 5500 bacterial strains have been isolated and identified. Here we present bacteriological data from day 1 samples. The number of patients with acute otitis media was 185 and the patients with acute upper respiratory tract infection without AOM was 103. The composition of bacterial flora in both groups is shown on figure 1.

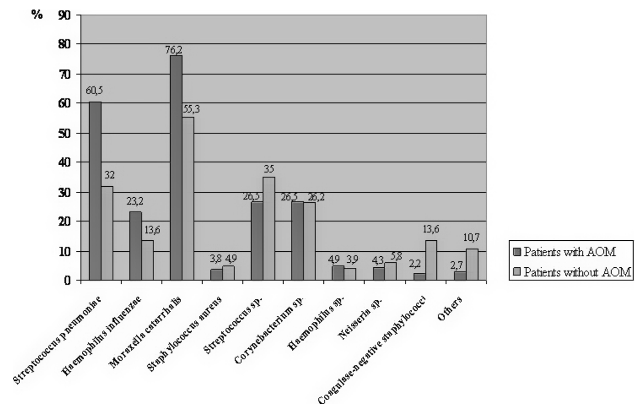

Figure 1. Bacterial flora in nasopharynx of patients with acute otitis media (AOM) vs. patients with acute upper respiratory tract infection without AOM.

The most common bacterial pathogens were *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis*. The numbers of *Streptococcus pneumoniae* and *Moraxella catarrhalis* in AOM group were significantly (P < 0.01) higher than in a non-AOM group.

Conclusion. On day 1, the composition as well as the quantity of bacteriological findings correlates with the diagnosis. The unique antibiotic vs. placebo study and quantitative culture provides opportunity to follow both the qualitative and quantitative changes of microbiota during convalescent phase of otitis media. After the study drug code is opened also the long term effects on microbiota can be evaluated.

P2199 Molecular biological study of infectious mechanisms in conjunctivitis, otitis media, and rhinosinusitis in young children

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Objectives: Acute conjunctivitis together with acute otitis media and acute rhinosinusitis are common bacterial infectious diseases with a high incidence during infancy and childhood. The present study was designed to find infectious mechanisms in this disease complex by use of bacteriological and molecular-biological techniques.

Methods: The subjects of this study were children at ages 60 months or less who visited our clinic, with nasal discharge and ear symptoms and/or conjunctivitis. Their nasal discharges (ND), conjunctival lavage fluids (CLF) and middle ear fluids (MEF) were subjected to bacterial culture. The antibiotic susceptibilities and PCR-based genotype of isolated bacteria were examined. When the pairs of bacteria isolated from ND and MEF or CLF were thought to be identical, the genomic polymorphysm were further analysed by pulsed-field gel electrophoresis (PFGE).

Results: The ages of 170 subjects ranged from 5 to 48 months (mean: 21.3 months). A total 172 *S. pneumoniae* was isolated from patients with conjunctivitis and rhinosinusitis and/or otitis media. Seven (7.2%) isolates from ND and 5 (9.8%) isolates from CLF were gPRSP strains. A total 294 *H. influenzae* was isolated from patients with conjunctivitis and rhinosinusitis and/or otitis media. Thirty-five (26.9%) isolates from

ND, 32 (27.8%) from CLF and 10 (18.2%) from MEF were gBLNAR strains. All 12 pairs of *S. pneumoniae* from ND, CLF, and MEF showed identical clones. Among 31 pairs of *S. pneumoniae* from ND and CLF, 29 pairs (93.5%) showed identical clones of *S. pneumoniae*. All 13 pairs of *S. pneumoniae* from ND and MEF showed identical clones.
Conclusion: In the present study, it is noteworthy that BLNAR strains, in which PBP3 mutation is responsible for development of resistance (MIC of ampicillin $\geqslant$4 µg/ml), accounted for 27.8% of all *H. influenzae* strains isolated. Based on the fact that PFGE revealed 98.9% of patients possessed indistinguishable clone of *H. influenzae*. Bacterial isolates from CLF and MEF in younger children were found to be identical with those from nasal discharge by the molecular-biological analysis. These results strongly suggest that bacteria in nasopharynx and nasal discharge will cause otitis media and conjunctivitis by ascending infections via Eustachian tube to middle ear cavity resulting otitis media and via naso-lacrimal duct to conjunctiva resulting conjunctivitis.

P2200 Procalcitonin in the diagnosis of acute bacterial otitis media

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Objective: PCT has been used for differentiating clinical syndromes that may be associated with bacterial infection from those that are not. The accuracy of PCT as a diagnostic test to differentiate between bacterial and non-bacterial AOM was studied in an investigator-blinded dose ranging study of faropenem medoxomil in AOM.
Methods: 328 subjects aged 6 months to <7 years were enrolled in a double tympanocentesis study of AOM. 277 subjects were analysed after excluding those who had received antibiotics in the preceding 7 days and those missing a baseline PCT assay. Subjects were evaluated using the Dagan severity of symptoms score consisting of scores of 5 signs and symptoms with a possible maximum score of 15. A PCT value of 0.5 ng/ml was used as a cutpoint indicating bacterial infection.
Results: 164 subjects (59%) had single or mixed bacterial pathogens cultured from middle ear fluid. PCT levels in the different groups (Table).

Organism	N	Mean Dagan Severity score	Mean (±S.D.) Procalcitonin	n > 0.05 (%)	N >0.5 (%)
No Growth	113	9.50	0.12 (0.43)*	34	4
S. pneumoniae	66	10.12	0.14 (0.35)	41	7
H. influenzae	53	9.53	0.08 (0.14)	32	4
M. catarrhalis	16	9.13	0.07 (0.13)	25	6
S. pyogenes	11	10.09	0.03 (0.02)	18	0
Mixed infections	18	9.28	0.13 (0.19)	39	6

*Excluding single outlier procalcitonin of 4.39, mean (SD) for no organism group was 0.09 (0.15).

There was no difference in mean Dagan scores or PCT levels between bacterial and non-bacterial AOM. The mean PCT concentration was higher in *S. pneumoniae* and mixed infections vs. non-bacterial AOM, but this did not reach statistical significance. At cutpoints of 0.05 or 0.5, the likelihood ratio of a positive test indicating a *S. pneumoniae* infection (vs. non-bacterial infection) is 1.2 and 3.5, respectively. The sensitivity and specificity are 41% and 66% for 0.05 and 7% and 98% for 0.5 cutpoints. Mean PCT concentration was more than halved at TOC regardless of bacteriologic or clinical outcome.
Conclusion: PCT was unable to differentiate bacterial from non-bacterial AOM, even when the most inflammatory infection due to *S. pneumoniae* is the cause. This may be due to the lesser degree of inflammation with AOM compared with sepsis or pneumonia. PCT level cannot be used to decide upon initiation of an antibiotic to treat AOM.

P2201 Rapid diagnosis of *Streptococcus pneumoniae* serotype 1 by real-time PCR

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Streptococcus pneumoniae serotype 1 is an important cause of invasive pneumococcal disease (IPD) and can produce epidemic outbreaks. Recently, emergence of IPD (particularly empyema), caused by serotype 1 has been reported in paediatric patients. Serotyping based on the quellung reaction requires substantial training and time.
Objective: The purpose of this study was to develop a Real-Time PCR for rapid diagnosis of *S. pneumoniae* serotype 1 in direct samples of patients with IPD and healthy carriers.
Methods: This is a prospective study including all paediatric patients with IPD who were diagnosed by culture or pneumolisin-Real-Time PCR at our institution (January–November 2007). In addition, we also included the results of oropharyngeal swabs of a cohort of 65 healthy children studied during the same period. Real time PCR of ugd (cpsk) gene specific of serotype 1 was performed in direct samples, and the time of detection was less than 3 hours.
Results: We included a total of 71 episodes of IPD in 69 paediatric patients (range age 2 months-17 years, mean age 4 years). 30 episodes were positive by culture and Real-Time PCR and 41 only by Real-Time PCR. Serotype 1 accounted for 26% of episodes identified by culture (8 of 30 episodes) and 24% of episodes identified only by Real-Time PCR (10 of 41 episodes, p=NS). All 18 children with IPD caused by serotype 1 were diagnosed of pneumonia (16 of them with empyema). Among 65 healthy children in whom oropharyngeal swabs were cultured, 17 (26%) were pneumococcal carriers, and serotype 1 was identified in only 1 case; specific serotype 1 Real Time PCR did not identify any additional case. All pneumococcal strains of serotype 1 were susceptible to penicillin and Erythromycin.
Conclusion: Real Time PCR allows a rapid identification of *Streptococcus pneumoniae* serotype 1. Since this serotype is not included in the current licensed 7 valent-pneumococcal conjugate vaccine (PCV7), rapid identification of epidemic serotypes (like serotype 1) is mandatory to implement other preventive measures and control outbreaks.

P2202 Nasal carriership of *Staphylococcus aureus*, *Streptococcus pyogenes* and *Streptococcus pneumoniae* in children visiting daycare centres

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Staphylococcus aureus (SA) and *Streptococcus pyogenes* (SPy) can cause significant morbidity in children (e.g. impetigo). *Streptococcus pneumoniae* (SPn) is the most common cause of bacterial meningitis in adults and children. In the Netherlands neonates are vaccinated for SPn since April 2006. In this study the (risk factors for) carriership of SA, SPy and SPn in children visiting daycare centres were determined.
Cross-sectional study between March and July 2007 in which nasal swabs and questionnaires (including socio-demographics, as well as some behavioral characteristics) were taken from 620 children (aged 0–4 years) in 48 randomly chosen daycare centres in the southern part of the Netherlands. Identification of SA, SPy and SPn was performed using standard biochemical tests. Susceptibility for fucidic acid was determined using the disc diffusion method. Logistic regression analyses were done to identify independent risk factors for carriership ($p < 0.1$).
A nasal carriership of 17.3% was shown for SA, 0.8% for SP and 37.8% for SPn. MRSA was not found in any tested sample. 5.7% of the SA positive samples were resistant and 23.5% were intermediate susceptible for fucidic acid, the standard treatment. Only 0.9% were resistant of mupirocine, the second choice treatment.
Preliminary analyses on 371 subjects revealed that independent predictors for presence of SA were young age ($p = 0.09$) and skin diseases (mostly eczema, $p = 0.01$), but this was only the case for children

under the age of 2. Carriership of SPy and SPn were inversely related ($p < 0.001$). Two respondents who reported keeping pigs were both carrier of SA ($p = 0.04$). Respondents keeping farm-animals tend to be less often sensitive to fusidic acid ($p = 0.09$).
The absence of SA was the only predictor for carriership of SPn. Carriership of SPn is not different between children who received (at least one) SPn vaccination and non-vaccinated children.
Nasal carriership of SA in children is slightly lower than the carriership in the common population (20–55% in the Netherlands). A high percentage of carriers are diminished susceptible for the standard treatment of impetigo caused by SA. Further research on the carriership and transmission patterns of SA is currently being conducted.
We showed that SPn can be successfully obtained from nasal swabs.
Results further suggest that SPn vaccinated children are still part of the SPn transmission chain.

P2203 Biofilm formation of *Streptococcus pneumoniae* isolates from nasopharynx and middle-ear effusion

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Objectives: We aimed to evaluate the biofilm formation of pneumococci in vitro in a large number of clinical isolates obtained from middle ear effusion and nasopharynx.
Methods: In total 153 clinical isolates from children attending an otitis media prevention trial were used. All isolates were serotyped at the National Public Health Institute in Oulu, Finland. Bacteria were grown in BHI until mid-logarithmic phase and diluted 1:100 in BHI or in BHI supplemented with 0.5% glucose or 0.5% fructose. Two hundred ul of diluted bacterial suspension was incubated in triplicate on a polystyrene plate for 18 h at 37ºC with 5% CO2. After removal of supernatant and after crystal violet stain (0.4% 50 ul 15 min) wells were washed three times with PBS to remove unattached cells. Optical density was measured at 540 nm after dissolving biofilm in 50 ul of DMSO. To validate the method we used scanning electron microscopy to visualize the structure of biofilms on bottoms of polyvinyl plates. Quantitative PCR was used to measure the number of pneumococcal genomes in biofilm.
Results: Altogether 62 of 153 (41% strains from nasopharynx and middle ear fluid were good biofilm producers (the mean OD >0.5) in nutrition-rich environment after supplementing BHI with 0.5% glucose or 0.5% fructose. The ability to form biofilm in vitro varied among pneumococcal isolates between different serotypes. Pneumococcal serotypes that most effectively produced biofilm were 6A, 14, 6B, 19A, and 19F. The site of isolation did not affect the ability of pneumococcal strains to form biofilm in vitro. In BHI supplemented with 0.5% glucose the OD value of pneumococcal isolates from middle ear effusion was 0.47 (SD 0.11) and 0.50 (SD 0.13) in isolates from nasopharynx (95% CI of the difference –0.08 to 0.021).
Conclusions: The ability of pneumococcal strains isolated from nasopharynx and from middle ear effusion of children to form biofilm in vitro was common. The serotype of pneumococcal isolate seemed to affect its ability to form biofilm. The serotypes that most effectively produced biofilm in our study (6A, 14, 6B, 19F) are among the pneumococcal serotypes that most often cause pneumococcal otitis media in children in our country.

P2204 Invasive pneumococcal disease in children after introduction of pneumococcal conjugate vaccine

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Objectives: Determination of antibiotic susceptibility and serotype distribution causing invasive pneumococcal disease (IPD) in children after comercialisation of the 7- pneumococcal valent conjugate vaccine (7PCV).
Methods: We analysed the clinical and microbiological data from paediatric patients with invasive strains of *S. pneumoniae*. We included all paediatric patients with isolation of *S. pneumoniae* in blood, cerebrospinal fluid or pleural fluid. Bacterial identification testing was performed using the VITEK 2 automated system (bioMerieux, France). Serotyping and antibiotic susceptibility were conducted in The National Reference Laboratory of Pneumococci, at the Nacional Center for Microbiology of Majadahonda, Madrid. Antimicrobial susceptibilities were interpreted according to Clinical Laboratory Standards Institute (CLSI) methodology and interpretive meningitis breakpoints.
Results: From January 2005 to December 2006, a total of forty-four *S. pneumoniae* invasive isolates were recovered from clinical specimens: 38 blood, 5 cerebrospinal fluid and 1 pleural fluid. Overall, 79.54% isolates were serotypes no contained in 7PCV. The most frequently isolated serotypes were 1 (9/44), 19A (9/44), 7 (6/44), 5 (5/44). The others 15 isolates were divided among the remaining serotypes 6A, 23F, 9V, 6B, 35F, 15C, 10A, 18C, 16, 14, 19F.
The main clinical manifestations included sepsis/bacteraemia (15.9%), pneumonia (50%) and meningitis (18.18%).
One case of meningitis, among NVS group, showed intermediate resistance to cefotaxime.
Isolates in NVS group showed a low level of resistance to penicillin (resistant 2.85%, intermediate 8.57%, susceptible 88.57%) and cefotaxime (resistant 0.0%, intermediate 5.71%, susceptible 94.28%) and moderate resistance to erythromycin (resistant 17.14%, intermediate 0.0%, susceptible 82.85%).
The VS group showed reduced susceptibility to penicillin (resistant 22.22%, intemediate 44.44%, susceptible 33.33%) and cefotaxime (resistant 0.0%, intermediate 22.22%, susceptible 77.78%) and high resistance to erythromycin (resistant 44.44%, intermediate 0.0%, susceptible 55.55%).
Conclusions: Our findings suggest a replacement of vaccine serotypes (VS) by nonvaccine serotypes (NVS) betwen the distribution of pneumococcal serotypes causing IPD in paediatric patients.
NVS isolates showed a lower level of resistance to antibiotics used as first choice treatment in pneumococcal invasive disease, penicillin and cefotaxime, than VS.

P2205 Nasopharyngeal carriage of *Streptococcus pneumoniae* in healthy Venezuelan children

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Objective: To determine the prevalence of, risk factors and serotype-distribution of pneumococcal carriage in healthy Venezuelan children in Caracas, with emphasis on the theoretical coverage of the 7-valent pneumococcal conjugate vaccine.
Methods: Nasopharyngeal swabs were taken from 557 healthy children aged 3–36 months attending the Healthy Children Service at Hospital de Niños "J.M. de Los Rios" in Caracas between December 2006 and August 2007. The swabs were cultured for *Streptococcus pneumoniae*, the isolates were serotyped and their susceptibility for penicillin, erythromycin, clindamycin, tetracycline, levofloxacin, vancomycin, trimethoprim/sulfamethaxazole and linezolid assessed by disk diffusion. Risk factors such as care centre attendance, young age, recent antibiotic use, race, current otitis media and social level were analysed by univariate and multivariate logistic regression analysis.
Results: In total, 111 pneumococcal isolates were collected. The overall carriage rate for *S. pneumoniae* was 20%, not significantly varying with age or time of year the sample was taken. The most important capsular serotypes/serogroups were 6 A and B (42%), 14 (11.3%), 23F (8%), 19A (6.5%) and 19F (5%). The theoretical coverage of the 7-valent pneumococcal conjugate vaccine was 74% (including cross-reactive serotypes). 45% of the pneumococcal isolates were resistant to one or more antibiotics. The rate of penicillin and macrolide resistance were 30% and 28%, respectively. Multi-drug resistance was found in 25% of the strains. No risk factors for pneumococcal carriage were identified.
Conclusions: The prevalence of pneumococcal colonisation in these children was low (20%) and didn't vary with age. A possible reason

for the low carriage rate might be the fact that this epidemiological survey addressed only healthy children. The serotypes most frequently found in our healthy population (6 A/B, 14, 23F, 19A and 19F) are those commonly involved in invasive pneumococcal diseases in Venezuela, highlighting the importance of nasopharyngeal carriage in the development of severe infections. The theoretical coverage of the 7-valent pneumococcal conjugate vaccine was high. None of the risk factors described in previous studies were found to be related to pneumococcal colonisation in this population. However, this is an ongoing study and the small number of carriers found up to now is limiting the statistical power of univariate and multivariate analysis.

P2206 Prevalence of multidrug-resistant *Streptococcus pneumoniae* in children with community-acquired pneumonia

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Objective: To evaluate the incidence of pneumococcal infections in children with community acquired pneumonia (CAP), the serotype and the antimicrobial susceptibility.
Material and Methods: During a three year period (2005–2007) 450 children with bacterial CAP were hospitalised. A total of 240 *Streptococcus pneumoniae* (S.pn) strains were isolated from 100 purulent sputum (41.6%), 80 bronchoalveolar fluid (33.3%), 42 pleural effusions (17.5%) and 18 blood (7.5%). Identification and susceptibility testing (MICs) were performed by VITEK 2 (bioMèrieux). Serotyping of S.pn isolates was carried out by multiplex-PCR assay at the National Meningitis Ref. Laboratory.
Results: Bacteraemic and non bacteraemic pneumococcal pneumonia occurred in 240 cases, 53.3% (males 54.1%-females 45.9%). The incidence of CAP, peaks between winter and early spring months. A total of 39.1% S.pn strains were considered to be susceptible to penicillin (PSSP), 37.6% showed intermediate resistance (IM) and 23.3% high level resistance to penicillin (PNSP), (MIC50,90 0.5–>2 mg/l). Increased resistance was also observed to cotrimoxalole (SXT) 37.9% (MIC50,90 0.5–>8 mg/l), tetracycline 36.6%, Quinopristin/Dalfopristin 9.1% (MIC50,90 0.5–2 mg/l). Lower resistance was found to third generation cephalosporins 8.3% (MIC50,90 0.25–2 mg/l) and chloramphenicol 4.17% (MIC50,90 0.25–4 mg/l). Significant resistance was observed for erythromycin (IM 5%, HLR 40%; MIC50,90 (0.5–>1 mg/l) and clindamycin 16.6. All S.pn strains were susceptible to newer quinilones, linezolide and vancomycin. The M-phenotype accounted for 55.5% of the erythromycin-resistant isolates. The overall multidrug resistance (MDR) was 28% (PNSP 74.6%, PSSP 25.3%). The most prevalent serotypes were: 19F, 6B, 14, 19A, 3 and a high proportion were of not typable (not included in the 7-valent conjugate vaccine).
Conclusions. S.pn remains the leading cause of CAP in children less than 3 years old. The appearance of MDR among PNSP isolates probably complicate treatment of severe CAP with empyema. A significant proportion of serotypes causing CAP are not included in the heptavalent vaccine.

P2207 The cost-effectiveness of palivizumab for respiratory syncytial virus prophylaxis in premature infants with a gestational age of 32–35 weeks in Canada

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Prophylactic therapy with palivizumab, a humanised monoclonal antibody, reduces the number of respiratory syncytial virus (RSV)-related hospitalisations in preterm infants, including those in the 32 to 35 weeks gestational age (GA) subgroup. The cost-effectiveness of this therapy from the payer and societal perspectives in Canada is unknown.
Objectives: To evaluate the cost-effectiveness of palivizumab as respiratory syncytial virus prophylaxis in premature infants born at 32 to 35 weeks GA.
Design: A decision analytic model was designed in this subgroup of premature infants to compare both direct and indirect medical costs of the patient with future lost productivity and benefits of prophylaxis. Four types of sensitivity analyses were performed to ascertain the robustness of the model based on mortality, health utility scales, variable discounting rates and administration costs.
Setting: Canadian publicly funded healthcare system (base-case analysis).
Participants: Canadian infants born at 32 to 35 weeks gestation without chronic lung disease.
Interventions: Palivizumab prophylaxis versus no prophylaxis.
Main outcome measures: Expected costs and incremental cost-effectiveness ratio expressed as cost per quality-adjusted life-year (QALY) gained using $ Canadian (CAD) 2006.
Results: The expected costs were higher for palivizumab prophylaxis as compared with no prophylaxis. The incremental cost-effectiveness ratio for the base-case scenario was $16,605 per QALY after discounting, which is considered highly cost-effective. The model was not sensitive to variation in the mortality rate associated with RSV. Sub-analyses that varied the number of risk factors in a Canadian validated risk scoring tool (Fig 1) were sensitive to the resulting variation in RSV-related hospitalisation rates. In instances where one risk factor or less was present, palivizumab was not cost-effective. However, for infants with two or more risk factors, or at least moderate risk (Figure 1), palivizumab had incremental costs per QALYs that indicated moderate to strong evidence for adoption (range: –$3642 [cost savings] to $77,495 per QALY).
Conclusions: Palivizumab was cost-effective and our model supports prophylaxis for infants born at 32 to 35 weeks GA, particularly those with two or more risk factors.

QUESTION	ANSWER	
	YES	NO
Born in Nov, Dec, or Jan	25	0
Infant or siblings in daycare	17	0
>5 individuals in the home including the infant	13	0
Small for GA	12	0
Immediate family history without eczema	12	0
Male	11	0
>2 smokers in the home	10	0
TOTAL SCORE:		
Low Risk Score: 0-48; Moderate Risk Score: 49-64; High Risk Score: 65-100		

Figure 1. Risk assessment tool.

P2208 Clinical, epidemiological and virological features of an aseptic meningitis outbreak in northeastern France, 2005

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Introduction: The frequency of aseptic meningitis outbreaks is increasing worldwide and enteroviruses (EVs) are now considered as a major viral aetiological cause of this neurological syndrome in paediatric patients.
Objectives: To assess the clinical, epidemiological and virological features of an aseptic meningitis outbreak in North-East of France, 2005
Patients and Methods: From May to November 2005, we retrospectively selected 80 children hospitalised in the North-East of France (Sex ratio M/F: 2.64; median age: 7.5 years (range 1 month-17 years)) who demonstrated classical clinical or biological signs of aseptic meningitis. Classical bacterial and virological culture assays (cell culture and seroneutralisation assays) were performed on cerebrospinal fluid (CSF), throat and faeces samples. Herpesviridae multiplex and enterovirus PCR assays were performed on CSF samples of the study patients. In cases of EV positive CSF samples, a phylogenetic comparison of partial EV VP1 capsid protein region was performed.
Results: A typical aseptic meningitis syndrome was diagnosed in 2/3 of study cases. A fatal leukoencephalitis was developed in 18 months infants during the outbreak. No other atypical neurological syndromes was observed during the study. EVs were identified as aetiological cause of aseptic meningitis in 73 of 80 (91%) cases, whereas HHV6 and VZV were identified in 3 (4%) of study children. ECHOvirus 30, 18, 13, 6, 3 and Coxsackievirus A16 were the most frequently identified EV strains

(84%, 6%, 2%, 4%, 2% and 2%, respectively). Phylogenetic analysis revealed that our ECHOvirus 30 strains were genetically closer to those isolated during 2000 aseptic meningitis outbreak comparatively to those identified during 2004 and 2006 non epidemic years. Moreover, our genetic analysis indicated the co-circulation of two distinct ECHOvirus 30 variants during the 2005 outbreak.

Conclusion: ECHOvirus 30 is actually the most common enterovirus strain involved in aseptic meningitis outbreaks. The present study highlights the need for a sentinel laboratory network for a national surveillance of clinical, epidemiological and virological features of EV paediatric infections. The potential genetic drift or recombination of EV genomic RNA could be responsible for the emergence of new potential epidemic strains associated with new neurological syndromes.

P2209 Impact of real-time polymerase chain reaction in the diagnosis of acute meningitis in children

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Introduction: Meningitis is one of the most important infectious syndromes in medical emergencies in hospitals, due to its high morbidity and mortality. Meningitis caused by viruses normally present milder symptoms and are the most common, however bacterial meningitis causes greater mortality. Both aetiologies must be quickly distinguished in order to manage patients properly.

Objectives: To compare real-time polymerase chain reaction (PCR) with traditional cell and bacterial cultures in the diagnosis of acute meningitis.

Materials and Methods: 173 cerebrospinal fluid samples (CSF) and blood culture samples from children aged 55 days to 14 years old, collected from January 2006 to November 2007, were tested in the Microbiology Unit of Clinic Hospital of Valencia (Spain).

All samples were cultured for bacterial and viral detection following the laboratory standard procedure. Chocolate agar, Sabouraud-chloramphenicol agar and Brain Heart Infusion broth were used for bacterial culture; and for viral detection the samples were inoculated in RD, VERO and MRC-5 cell cultures.

Nucleic acid was extracted using a BioRobot EZ1 Workstation with EZ1 Viral Mini Kit v2.0 (Qiagen) and with EZ1 DNA Blood 200 μl Kit (Qiagen) for RNA and DNA respectively.

Multiplex real-time PCR was used to test each sample for *Neisseria meningitidis* and *Streptococcus pneumoniae* using Realcycler MENE-01 (Ingenie Molecular) in the Smart Cycler® II System (Cepheid). Enteroviruses were tested using real-time reverse transcription-PCR with the Enterovirus Analyte Specific Reagent kit and the Smart Cycler® II System or Xpert EV kit and a GeneXpert® thermocycler (Cepheid).

Results: Fifty-three patients (30.6%) were positive, 44 of them were positive for enterovirus; 35 cases were positive only by PCR, 4 only by cell culture, and 5 by both methods. Sensitivity was 90.9% for the real-time PCR method but only 20.4% for cell culture.

PCR method was able to detect 6 patients infected with *N. meningitidis* and 3 with *S. pneumoniae*, whether by culture only 4 cases for *N. meningitidis* (only 1 from a CSF sample) and 1 case for *S. pneumoniae* (by blood culture) were detected. PCR showed 100% sensitivity when compared to CSF culture (11%) and blood culture (44%).

Conclusion: Real-time PCR has shown to be a powerful and rapid diagnostic tool in acute meningitis; bacterial and viral aetiologies were characterised in 4 to 5 hours, helping to reduce antibiotic treatment and hospitalisation time.

P2210 Evaluation of new enzyme immunoassays for the detection of meta-pneumovirus infection in a paediatric population

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Objectives: To evaluate the effectiveness of a new enzyme immunoassay assays: hMPV EIA (Biotrin International Ltd., Ireland) in the diagnostic of metapneumovirus infection in children, comparing the results with a molecular assays: real-time RT-PCR (Pro hMPVTM Real Time Assay Kit. Prodesse).

Methods: 105 nasopharyngeal specimens were collected from 103 children less than 5 years of age hospitalised with symptoms consistent with viral respiratory infection. Samples were obtained at Paediatric Service at the University Hospital Germans Trias i Pujol, during october-april 2007. The assays were performed according to the recommendations of the manufacturer. Sensitivity, specificity, correlation and kappa values were calculated. Clinical dates of the patients were recorded. Disease severity was determined by the required oxygen supplementation during the hospitalisation.

Results: EIA detected antigen metapneumovirus in 11/105 samples (10.5%). In one case the results was uninterpretable. Real-time RT-PCT was positive in 11/105 cases (10.5%). In 14 cases the reaction was inhibited.

In 8 cases both tests were positive. The concordance between the EIA test and real-time RT-PCR with a interpretable results was 94.5% (86/91) (k =0.731).

The percentage of children who required oxygen therapy was higher in children infected with hMPV that if there was no infection by the virus. The children with hMPV infection required oxygen supplementation more frequently than did children not infected with hMPV ($p < 0.036$).

Conclusion: The results of hMPV EIA compared with the detection of metapneumovirus by real-time RT-PCR to indicate a good correlation between the two techniques.

The technique hMPV EIA is a good alternative for the diagnosis and management of metapneumovirus respiratory infections in paediatric populations.

P2211 Human Metapneumovirus and human Bocavirus in hospitalised children with acute respiratory infections

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Objectives: To determine the contribution in hospitalisation of both Human Metapneumovirus (hMPV) and Human Bocavirus (hBoV) infections among children younger than 3 years of age a retrospective study was performed. We collected and analysed respiratory samples from a paediatric population hospitalised at the San Carlo Borromeo hospital in Milan (Italy) between 2004 and 2007.

Methods: The study involved 186 (104 M, 82 F, mean age: 8.1 months) hospitalised children with acute lower respiratory symptoms (bronchiolitis, pneumonia and asthma). Informed consent was obtained from the parents of all the children who provided respiratory samples.

Pharyngeal swabs (PS) were collected from each patient and a form, containing information on demographic data, clinical diagnosis and epidemiological information, was filled.

HMPV-RNA and hBoV-DNA were extracted from PS and viral genome targets were detected by the amplification of 151bp of hMPV M gene and 354bp of hBoV NP gene.

Results: Overall, hMPV-RNA was detected in 19/186 (10.2%) PS samples and hBoV-DNA in 17/186 (9.1%) PS samples.

hMPV and hBoV infections were detected along with other respiratory viruses (Rhinovirus, Coronavirus 229E, Respiratory Syncytial Virus, Influenza A): the rate of co-infection was 26.3% for hMPV and 47.1% for hBoV. Ruling out the patients with co-infections, symptoms and clinical data were similar among hMPV and hBoV-infected children, even though 50% of hMPV-positive patients showed bronchiolitis.

Neither hBoV infections nor hMPV infections were identified in children over 2 years of age. Moreover, hMPV infection was more frequently observed in children with age $\leqslant 6$ months respect to older children ($p < 0.05$). No differences of age profiles were observed in patients with hBoV infection.

The overall rate of hMPV and hBoV detection in respiratory samples varied slightly from year to year, although without any significant difference.

When the respiratory samples were stratified by month of collection, a statistical variation of the hBoV impact was observed. In fact, the highest

frequency of hBoV (47.1% of total hBoV-positive samples) was detected in January. No seasonal variation in hMPV frequency was noted.

Conclusions: HMPV and hBoV has been associated with acute respiratory infection in young children. Our data suggest that both viruses are involved in episodes of hospitalisation in the paediatric population younger than 3 years of age with a similar frequency.

P2212 Comparison of prevalence and clinical features of paediatric respiratory infections due to respiratory syncytial virus and human metapneumovirus

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Objectives: The purpose of this study was to compare the prevalence and clinical features of two paediatric respiratory pathogens, respiratory syncytial virus (RSV) and human metapneumovirus (hMPV) in children <5 yr.

Methods: Respiratory specimens (n = 657) collected 10/2004–4/2006 were tested for RSV and hMPV using NucliSENS analyte specific reagents and the NucliSENS EasyQ Basic Kit (bioMérieux, Durham, NC). Prevalence and admission rates were based on the total group. Chart review (n = 64) was conducted for 35 RSV+, 26 hMPV+, 3 RSV+/hMPV+ children evaluated in the ED or hospitalised. Case report forms were used to evaluate patient demographics, clinical findings, and clinical course.

Results: The overall infection rates were: RSV= 22.5% (<2 yr: 27.5%; 2–<5 yr: 12.7%), hMPV= 5.5% (<2 yr: 5.8%; 2–<5 yr: 4.6%). RSV and hMPV seasons overlapped although RSV peaked Nov-Jan and hMPV peaked Feb-April. The percentages of children requiring hospitalisation were 24.5% (RSV+) and 58.8% (hMPV+). Patients with chart review showed similar age distribution for RSV+ and hMPV+ (<2 yr: 65.7% vs 65.4%, 2–<5 yr: 34.3% vs 34.6%, respectively). There was no statistically significant difference in underlying co-morbidities, which included reactive airway disease, significant prematurity, muscular dystrophy, chronic lung disease, congenital heart disease, and leukaemia (p = 0.4004). Clinical findings were similar in both groups in terms of retractions, wheezing, and crackles (p = 0.2300, p = 0.9839, p = 0.3425, respectively). hMPV+ patients were more likely to present with fever ($p \leqslant 0.001$) and rash (p = 0.0111). RSV+ patients were more likely to present with rhinorrhoea (p = 0.0033) and diarrhoea (p = 0.0340). Although not statistically significant there was a trend towards more chest radiographs in hMPV+ patients (p = 0.0553). Upper and lower respiratory tract infections were seen in all groups but 19.2% of hMPV+ patients required mechanical ventilation versus 11.4% of RSV+ patients. This however did not affect length of stay in the intensive care unit or total hospital stay for either group. Patients co-infected with RSV and hMPV were not more likely to have severe disease.

Conclusion: RSV is the most prevalent cause of respiratory disease in children <5 yr. Seasonality and clinical symptoms of RSV and hMPV overlap, yet there are distinctions in prevalence peaks and clinical symptoms. This data suggests that hMPV infection may result in more hospital admissions and increased severity of symptoms.

P2213 A 1-year longitudinal study on the colonisation of five upper respiratory tract pathogens within a daycare centre

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Day-care centres (DCC) are settings where children are at increased risk for carriage of upper respiratory tract (URT) pathogens. Longitudinal studies on the dynamics of colonisation by such pathogens within the DCC are only a few and have often focused on a single pathogen. To better understand the patterns of colonisation by such pathogens, we conducted a large one-year longitudinal study – with 11 sampling periods – starting in February 1998 on nasopharyngeal carriage of *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Staphylococcus aureus* and *Streptococcus pyogenes* among a group of 47 children who attended a single DCC.

Methods: All isolates were antibiotyped. *S. pneumoniae* and *H. influenzae* were genotyped by PFGE. *S. pneumoniae* were also serotyped.

Results: A total of 414 samples were collected of which 83.3% had at least 2 of the 5 bacterial species and only 2.4% of the samples had none of them. All children were colonised by *H. influenzae* (with a minimum of 4 positive samples and a maximum of 11) and *M. catarrhalis* (minimum 2 positive samples, maximum 10). All but one child were colonised by pneumococci (maximum 10 positive samples), and 47% were colonised at some point by *S. aureus* being the corresponding figure for *S. pyogenes* 55%. Sixty-eight percent of all positive cultures yielded at least one isolate (*H. influenzae*, *M. catarrhalis*, or *S. aureus*) that produced β-lactamase; 24% of the pneumococci were not susceptible to penicillin; all *S. aureus* were susceptible to oxacillin, and 23% of *S. pyogenes* were resistant to erythromycin. Molecular typing showed the introduction of pneumococcal and *H. influenzae* clones during all year. Over 95% of all pneumococcal and *H. influenzae* isolates belonged to clones shared by more than one child. Children were sequentially colonised with up to 6 pneumococcal clones (mean 3.6) and 5 serotypes (mean 3.4); and 9 *H. influenzae* clones (mean 7.1). Clones with increased capacity for transmission and/or prolonged colonisation were identified.

Conclusions: Abundant co-colonisation by pathogens of the URT was found. Antimicrobial resistance was present in all species. Molecular typing showed a high rate of acquisition and turnover of pneumococci and *H. influenzae* strains and detected very high levels of cross-transmission. DCC are units where permanent introduction of clones occurs, and attendees are a pool of hosts where the fittest clones find privileged opportunities to persist and expand.

P2214 Myocarditis outbreak with fatal cases associated to Adenovirus subgenera C among children, Havana City, 2005

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Background: Acute myocarditis is characterised by inflammatory infiltrates of myocardium. Disease has been attributed to multiple causes, but viruses particulary Enteroviruses and Adenovirusrs are considered to be the leading causes of usually clinical myocarditis.

In the summer 2005 an outbreak of atypical febrile syndrome accompanied with acute cardiac decompensation among infants and young children was observed in Havana City. Eleven patients had a torpid evolution and eight fatal cases due to myocarditis were informed.

Methods: The aim of the present study was to investigate the aetiological agent. Two groups of children admitted in paediatric hospitals of Havana City from July 3 to August 2 were studied. Forty clinical samples (necropsy tissue, cerebrospinal fluid, stools, serum were collected and tested by molecular methods to detect 14 Respiratory Viruses, 6 Herpesvirus and generic Enteroviruses, and Flavirus and Alfavirueses. Virus isolation was performed in A-549 cells. Isolated viruses were typed by sequence analysis.

Results: Adenovirus genome was detected in 6 of 8 fatal cases, was more common detected in lung and myocardium 5, (62.5%) and 3(37.5%) respectively. In two patients was detected in both organs. Virus isolation was obtained in 5 patients. In the non fatal cases, Adenovirus was detected in all of them. Virus recover was successful in 2 cases. Viruses detected and isolated were typed as Adenovirus 5 in the fatal cases and Adenovirus 1 in the non fatal cases. None other viruses were found.

Conclusions: Our data suggest that Adenovirus was the aetiological agent implicated in this myocarditis outbreak, after others viruses were discarded.

P2215 Prevalence of gastroenteritis caused by rotavirus and adenovirus in a paediatric population in Athens, Greece

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Objective: Acute gastroenteritis (AGE) is a major source of morbidity in early childhood. Enteropathogenic viruses are regarded as particularly relevant causative agents. Our study was performed to estimate the prevalence of gastroenteritis caused by group A rotavirus and enteric adenovirus among young children during a two-year period (2006–2007) in the area of Athens.

Methods: We reviewed retrospectively the laboratory cards of children under five years old, who were tested for group A rotavirus and enteric adenovirus, the major enteric viral pathogens. The detection of the viruses was carried out according to standard methods using a commercial immunochromatography assay kit.

Results: A total of 1 228 patients, younger than 5 years of age seeking medical care for acute diarrhoea in our hospital, were enrolled in the study. Diarrhoea, vomiting and fever were the main clinical manifestations. All stool samples from inpatients were collected within three days of hospitalisation. They were all tested for rotavirus and adenovirus. Group A rotavirus was the most frequently detected (176/1 228, 14.33%), followed by enteric adenovirus (30/1 228, 2.4%). Dual infections were found in 2.66% of the positive samples. Group A rotavirus gastroenteritis occurred in 87 boys and 89 girls and adenovirus gastronteritis occurred in 12 boys and 18 girls (male/female ratio 1:2). The median ages were similar (10 months for rotavirus and 11 months for adenovirus, range 30d-5years). The majority of patients were less than 3 years old; 82% and 76% for rotavirus and adenovirus infection, respectively. Rotavirus infection was most common in the winter through early spring, peaked between January and May. Enteric adenovirus gastroenteritis occurred year-round. Hospital outbreak was not recorded. All cases were classified as community-acquired. The results from 2007 will be completed at the end of the year.

Conclusions: Rotavirus is a significant viral agent among children, especially those younger than three years. The overall incidence of viral gastroenteritis could have been higher than currently observed, if the included patients had been tested for astrovirus and calicivirus.

P2216 TCRalfabeta and TCRgammadelta T lymphocytes in severe infected premature neonates

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Background: The lower value of T cells in premature infants are considered to be a responsible factor for susceptability to severe early-onset infections (e-oi).

Aim: To evaluate the relationship between the occurence of severe e-oi on the % and number of TCRab and TCRgd in prematures regarding their sex, BW, GA and Apgar score.

Material: The study population: 50 (31 boys, 19 girls) preterm neonates, among them 27 infected (15 septic, 12 pneumonic, with BW ranged from 1000 to 2390g, mean 1717g, and GA from 26 to 37 weeks, mean 32) and 23 without infections (controls) with mean BW 1650g and mean GA 32 weeks. Birth asphyxia in 81% of infected and in 30% of noninfected prematures was noted. Cesarean section in 70%, infectious perinatal risk factors in 52% of all cases were stated. The most common bacteria isolated from blood in septic prematures were staphylococcal strains (9). In 2 cases *Str. agalactiae*, in 3 – *Klebsiella pneumoniae* and in 1 case E. coli were noted. 4 prematures with sepsis complicated by shock and DIC due to *Klebsiella* (2) and *Staph. epidermidis* (2) died. The flow cytometric immunophenotyping (BD) was performed to determine TCRab and TCRgd T lymphocytes.

Results: The mean % (58.0±12.8, range 24–83%) and mean number (3.0±0.9, range 1.591–6.095 g/l) of TCRab in infected prematures did not differ ($p > 0.05$) from the mean values in controls (mean 59.6±7.3%, range 45–76%, 3.330±1.463, range 1.586–6.733). The mean % of TCRgd (3.78±2.34, range 1–10%) was significantly ($p = 0.03$) higher in severe infected prematures than in controls (range 1–9%, mean 2.8±2.2%). In infected prematures with gastrintestinal disorders, significantly higher the mean % of TCRgd (5.1±2.6%) than in infected preterm without such disorders (2.7±1.5%) was stated. Birth asphyxia was connected with significantly higher mean % of TCRab (64.5±10.4 and 52.8±12.3%). There were positive correlations between the % of TCRab and BW ($r = 0.42$, $p = 0.002$) and GA ($r = 0.48$, $p = 0.032$) in infected prematures. Negative correlation between number of TCRgd and BW ($r = -0.12$, $p = 0.04$) and GA ($r = -0.2$, $p = 0.038$) was noted.

Conclusions: 1. Severe e-oi increase the % and number of TCRgd lymphocytes in preterm neonates. 2. Birth asphyxia may influence on increase of percentage of TCRab cells in severe infected prematures. 3. Increase of % of TCRgd lymphocytes in infected preterm neonates with gastrointestinal disorders may prove the important role of these cells in development of those abnormalities.

P2217 Persistence of *Escherichia coli* clones and resistance genes in recurrent urinary tract infections in children

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Objectives: We compared the molecular clonality of *E. coli* (Ec) from recurrent urinary tract infection (rUTI) episodes with stability of phenotypic antibiotic resistance and presence of gene cassettes. The association between the presence of sul genes and previous trimethoprim-sulfamethoxazole TS treatment was assessed.

Methods: Altogether 78 urinary Ec isolates were obtained during one year follow-up from 27 patients (pt) (aged 2m-180m, mean 44.8m) with 1–5 episodes after initial UTI. Strain clonality was detected by PFGE. MIC values to TS, sulfamethoxazole SX, ampicillin AM, cefuroxime XM, cefotaxime CT, gentamicin GM, ciprofloxacin CI were measured by E-test. Int1, sul1–3 genes were detected by PCR and compared with TS treatment data.

Results: In 21/27 pt (78%) the unrelated clonality of Ec strains was detected: in 11 persistent clonal strains (Cs) ($n = 24$), in 10 both Cs and individual strains (Is) ($n = 24$; 16, resp) and in 6 only Is ($n = 14$) were found. The pt with only Is were younger than others (med 7.5 vs 48m; $p = 0.03$). Ec resistance to TS, SX, AM, XM, CT, GM, CI was 33%, 78%, 40%, 17%, 3%, 2%, 0% resp. The phenotypic antibiotic susceptibility in Cs was more stabile compared to Is (OR, 8.7; 95% CI, 1.8–40.8) during different infection episodes.

The integrons were found from 55/78 strains. Int pos strains had higher MIC values to XM, CT and GM than int neg ones ($p < 0.01$; 0.03; 0.01). The presence of integrons was similar in Cs and Is. In half of the pt ($n = 14$) it did not change, in another 13 the loss of integrons was more frequent than capture (9/13 vs 2/13, $p = 0.01$).

Sul genes were found in 42/78 Ec strains, it was in accordance with phenotypic resistance to SX compared to gene neg ones ($p = 0.03$). The Is harboured more sul 1 genes than Cs ($p = 0.01$). Occurrence of sul genes was more stabile in pt with Cs compared to Is (OR, 4.4; 95% CI, 1.1–17.7). The Ec strains obtained after previous TS treatment had more frequently any sul genes than TS non treated ($p = 0.01$).

Conclusion: In children the majority of Ec rUTI episodes are due to individual persisting clones. The stability of antibiograms and persistence of SX resistance encoding sul genes may indicate putative relapses. Previous TS treatment is associated with corresponding resistance.

P2218 Low incidence of congenital cytomegalovirus infection in a large maternity hospital in Ireland

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Objectives: The aim of this study is to determine the incidence of congenital CMV (cCMV) infection in a large tertiary referral maternity

hospital in Ireland. There has been no Irish data reported on this subject previously.

Methods: The study comprised of two parts: (1) A retrospective review of laboratory confirmed symptomatic cCMV cases diagnosed over three years (April 2004 to May 2007); and (2) A prospective pilot cCMV screening programme was introduced following Ethics Committee approval. One thousand urine samples are being obtained from neonates born in the hospital, with parental consent, from November 2006 to March 2008. Samples are tested in pools of five, each pool being tested by real-time polymerase chain reaction (rtPCR) for the presence of CMV DNA. Individual aliquots from any positive pool are re-tested by rtPCR to identify the positive specimen. Repeat urine for culture and DEAFF test, plus serum for CMV IgM, and plasma for CMV DNA are tested to confirm positive rtPCR results.

Results: (1) Eight children were identified with symptomatic cCMV infection. During the study period there were 25,444 births, giving an incidence of symptomatic cCMV of 0.03%. At birth, symptomatic cCMV is expected to account for 5–10% of all cCMV cases. Based on our confirmed cases, therefore, we expect an incidence of 0.3–0.6% cCMV from the screening study, or between 3 and 6 cases per 1000 babies screened. (2) Four hundred infants have been tested to date; only one positive baby has been detected, yielding an incidence figure of 0.25% cCMV. The positive PCR result was confirmed with a positive urine culture and DEAFF test. Of note, the serum IgM was negative. This screening study is ongoing and we aim for completion in spring 2008.

Conclusions: The incidence of cCMV infection in the population studied is low when compared with the published literature (0.3–2.2%). This is on a background of low CMV seroprevalence in Irish pregnant women (30% CMV seropositive). Enhanced screening of greater numbers is required to accurately assess the burden of cCMV disease in Ireland.

P2219 Community-acquired *Pseudomonas aeruginosa* enterocolitis and sepsis in infants

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Objectives: *Pseudomonas aeruginosa* is an important opportunistic pathogen that often infects patients with chronic disease or immune-compromised status. It is a well-known aetiology of nosocomial infection but community-acquired infection has been rarely reported. *P. aeruginosa* is generally not considered as a cause of infectious diarrhoea. Here, we reported 18 cases of community-acquired pseudomonas enterocolitis and sepsis in infants.

Methods: There were 18 children with community-acquired *P. aeruginosa* sepsis at Chang Gung Children Hospital from July 2003 to September 2006. Their demographic data, clinical manifestations, laboratory data, antibiotic susceptibility and clinical outcome were collected and analysed. Immunologic studies were performed to screen the possible immunodeficiency. Pulse-field gel electrophoresis using Spe1 was performed for 14 isolates collected to determine their clonal relationship.

Results: All patients were previously healthy before the infection. The age distribution among them ranged from 4 to 25 months. Sixteen were under 1 year of age. Thirteen occurred during July to November. All patients had fever. Diarrhoea was observed in 17 patients and vomiting in 5. Concomitant fever and diarrhoea were observed in 14 patients. Thirteen patients developed ecthyma gangrenosum. Seizure occurred in 4 patients, two of whom had meningitis. Leucopenia was seen in 9. Thrombocytopenia was observed in 11. C-reactive protein level above 50 mg/dl was detected in 16. All isolates were susceptible to various anti-pseudomonas antibiotics. Fifteen patients received empirical antibiotics appropriately. Surgical intervention in abdomen was performed in 7 patients and ventriculoperitoneal shunt in 2. Sixteen patients survived, 2 were left with severe neurologic sequelae. Immunologically, 6 patients had transient hypogammaglobulinaemia. No specific T cell and B cell deficiency was detected. Pulse-field gel electrophoresis revealed no identical or closely related strains among these isolates.

Conclusion: *P. aeruginosa* can be a community pathogen to cause enterocolitis and sepsis in infants. In our cohort of patients, majority were under 1 year of age. No significant immunodeficiency was found. Bacterial isolates appeared diverse in PGFE.

P2220 Influence of antibiotics during delivery work on early onset sepsis

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Objectives: 1. To assess the incidence of early onset sepsis with positive culture during the period 1996–2006 compared to a historic period previous to implementation of prophylaxis during delivery in women carrying *S. agalactiae* (1988–1994). 2. To know the aetiology and the mortality in these patients during the period of study.

Methods: Retrospective study of newborn less than seven days old, attended in the Neonatology Unit during the period 1996–2006 with a diagnosis of sepsis/meningitis and a positive culture of blood and/or CSF. The isolated microorganism, pregnancy week of the mother at delivery and evolution of illness were recorded.

Results: Twenty-nine newborns with early onset sepsis and positive culture (22 of them born in our own hospital and seven coming from other centres) were attended in the Neonatal Unit. Isolated microorganisms were: 15 *S. agalactiae*, 8 *E. coli*, 2 L.monocitogenes, 1 *C. albicans*, 1 *E. faecalis*, 1 *P. aeruginosa* and 1 *S. pneumoniae*. Eleven of the 29 newborn were preterm (38%), less than 37 weeks of pregnancy. In 10 cases, mothers had received antibiotics during delivery work. Onset of clinical illness was detected during the first day of life in 65.5% of cases. Mortality was due to infection in 17.2% of cases (five babies, all of them less than 31 weeks of pregnancy) and the remaining 24 children had a good evolution without sequelae. During the historic period, the incidence of early onset sepsis with positive culture was of 2.2/1000 live births (43 internal cases per 19453 live births) and that due to *S. agalactiae* was of 1.3/1000 live births (26 internal cases). In contrast, during the studied period the global incidence was of 0.5/1000 live births, with 22 internal cases per 40129 live births ($P < 0.001$) and that due to *S. agalactiae* was of 0.27/1000 live births, with 11 internal cases ($P < 0.001$).

Discussion: The use of prevention strategies against neonatal sepsis during the last years has lead to a significant reduction ($P < 0.001$) of early onset sepsis both as a global fact (from 2.2 to 0.5/1000 live births) and as that due to *S. agalactiae* (from 1.3/1000 to 0.27/1000 live births). The two predominant infectious agents during the studied period were *S. agalactiae* (51%) and *E. coli* (27%). Mortality has been related to extreme prematurity.

P2221 A 4-year study on paediatric vulvovaginitis

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Objective: The purpose of this study is determining the aetiology of paediatric vulvovaginitis with inflammatory exudates in our environment.

Methods: A retrospective study on 0–14 years old girls showing genital exudates with inflammatory cells in Gram stain, where any germ from resident flora or with a known pathogenicity was isolated between 2004 and 2007. A microbiological study of vulvar (26), vaginal (340) and vulvovaginal (33) exudates was made. Exudates were seeded in blood agar, chocolate agar, Thayer-Martin agar, Gardnerella agar, Sabouraud-chloramphenicol agar and bouillon for *Trichomonas*. Microorganisms were identified following conventional methods.

Results: 399 girls were studied, out of which 177 (29.3%) were diagnosed with vulvovaginitis. 29.7% of vaginal samples, 30.7% of vulvar exudates, and 24.2% of vulvovaginal exudates were positive. Patients were 7 years old on average. Distribution by age group was as follows: 0–1 years old (19), 2–3 years old (15), 4–5 years old (19), 6–11 years old (35), 12–14 years old (29). Distribution by year was as follows: 2004 (40), 2005 (41), 2006 (17), 2007 (19). There were obtained

the following isolations: *C. albicans*, 42 (35.8%); *E. coli*, 29 (24.7%); *G. vaginalis*, 14 (11.9%); *C. glabrata*, 7 (6%); *S. pyogenes*, 4 (3.4%); *S. agalactiae*, 4 (3.4%); *P. mirabilis*, 3 (2.5%); *S. aureus*, 3 (2.5%); *H. influenzae*, 3 (2.5%); *Candida* sp, 2 (1.7%); *Corynebacterium* sp, 1 (0.8%); *E. cloacae*, 1 (0.8%); *P. aeruginosa*, 1 (0.8%); *M. morganii*, 1 (0.8%); *K. oxytoca*, 1 (0.8%); *T. vaginalis*, 1 (0.8%). There was not detected any *E. vermicularis* or *N. gonorrhoeae* infection. 87.2% of girls suffered from vulvovaginitis caused by resident flora. Yeasts were found in the exudates from 26 patients between 12 and 14 years old. Vulvovaginitis was specific in 12.8% of total cases – it always had a respiratory origin, except for one case having a sexual origin.

Conclusions: As in other studies, vulvovaginitis in our area is more common at prepuberal age, and vulvovaginitis with an unspecific origin is the most common cause of leucorrhoea amongst girls. Symptoms in minor cases of unspecific vulvovaginitis can be alleviated by improving personal hygiene habits. The most frequently isolated microorganism was *C. albicans* followed by *E. coli*, in most cases along with mixed flora. *Candida* vulvovaginitis is more common at prepuberal age, more than half of total patients belonging to that age group.

P2222 Prevalence of Rotavirus, Adenovirus, Norovirus and Astrovirus infections by rapid EIA and ELISA assays in hospitalised French children

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Introduction: Rotavirus, Norovirus, Astrovirus and Adenovirus 40/41 have been recognised as the most important aetiological agents of childhood viral gastroenteritidis in industrialised countries. Little is known about the number and the distribution of these viruses in hospitalised children.

Objectives: To determine the prevalence and the distribution of viruses responsible for gastroenteritidis in hospitalised children.

Patients and Methods: From January to October 2007, a total of 623 faecal specimens were routinely collected from children with or without gastroenteritidis signs and who were born or admitted in two French hospital settings (Reims medical university centre; n = 280; mean age, 2.18±SD 3.64 years, range: 2 days – 12 years; Brest medical university centre n = 343; mean age, 1.62±2.4years, range: 4 days – 14 years). Rapid EIA analyses for rotavirus and adenovirus were performed at the time of hospitalisation (r-Biopharm), and new commercially available ELISA tests were used retrospectively for the detection of Norovirus and Astrovirus in frozen stool samples (Ridascreen, r-Biopharm).

Results: The overall rates of prevalence for Rotavirus, norovirus, adenovirus, and astrovirus were respectively 29, 12, 4.5 and 2.5% and they did not significantly differ between hospitals (P = 0.09). Dual virus infections were detected in 30 (4.8%) of the 623 study children and were associated with Norovirus in 19 (63%) infants including 4 prematurity cases. During winter, Norovirus infections accounted for 33% of all hospitalised gastroenteritidis cases at a time where rotavirus was epidemic, resulting in mixed Norovirus and rotavirus gastrointestinal tract infections. Of the 299 documented viral gastroenteritidis, 9 (3%) were identified as nosocomial infections that 4 occurred in prematurity cases.

Conclusion: These findings provide evidence that Noroviruses can be a leading cause of gastroenteritidis, and highlight the need to implement norovirus and astrovirus ELISA detection assays in association with rapid EIA rotavirus and adenovirus EIA detection for the clinical diagnosis and the nosocomial prevention of gastroenteritidis viral infections in paediatric departments.

P2223 Enterococcal urinary tract infections in children

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Aim of this study was to evaluate the prevalence and resistance patterns of Enterococci in childhood nosocomial urinary tract infections (UTIs).

Material and Methods: A total of 2801 positive urine cultures (10.2%) were examined during a four year period (2004–2007). The study population was children aged 4 months to14 years (males 39%-females 61%). Urine cultures, identification and susceptibility were performed by conventional methods.

Results: Enterococci were isolated in 116 cases, accounting for 4.1% of the total UTIs. A percentage of 75.8% (88/116) strains were identified as *E. faecalis*, 14.6% (17/116) as *E. faecium* and 9.4% (11/116) as *Enterococcus* spp. The enterococcal UTIs in children less than 2 years old (median age 6 months) were associated with urinary tract instrumentation or structural abnormalities (.S.A). All *E. faecalis* isolates were resistant to quinupristin/dalfopristin (Q/D) (MIC50 4 mg/l-MIC90 8 mg/l) and susceptible to vancomycin (Va), teicoplanin (Teico), ampicillin (Am), amoxil-clavulanate (AMC) and linezolide (Li). In contrast *E. faecium* strains were significantly resistant to Am 94.1% (MIC50 16 mg/l-MIC90 32 mg/l), AMC 82.3% (MIC50 16 mg/l – MIC90 32 mg/l), Q/D 47% (MIC50 1 mg/l-MIC90 8 mg/l); lower resistance was found to Li 11.7% (MIC50 2 mg/l-MIC90 4 mg/l). The resistance rates of *E. faecalis* and *E. faecium* to quinolones were: Levofloxacin 7.41%-41.1%, ciprofloxacin 9.2%-64.7% and norfloxacin 11.4–58.8%. High level resistance (HLR) was detected to gentamicin and streptomycin in 15.9% and 36.3% of *E. faecalis* and in 35.2% and 70.5% of *E. faecium* respectively.. One *E. faecium* multidrug resistant strain, expressed the Van A genotype (VRE, Va >32 mg/l, Teico >32 mg/l) was isolated.

Conclusions: Enterococci were the fourth leading cause of UTIs in children. Infants less than 2 years old with Enterococcal UTI are highly indicative for S.A and recurences. *E. faecium* strains were considered to have multiple resistance phenotypes emerging a feared nosocomial pathogen while a significant persentage presented the unusual phenotype expressing susceptibility to Q/D. The overall proportion of vancomycin and linezolide resistant enterococcal isolates was found low in paediatric patients.

P2224 Group A *Streptococcus* virulence and host factors in two toddlers with rheumatic fever following toxic shock syndrome

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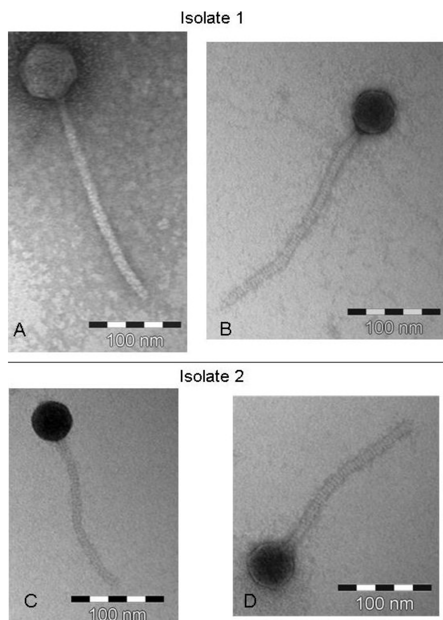
Objective: Rheumatic Fever (RF) 'classically' occurs after Group A *Streptococcus* (GAS) pharyngitis in children over 5 years in developping countries. GAS Toxic Shock Syndrome (TSS) is uncommon and does not usually lead to RF. Host-pathogen interactions are of utmost importance for the development of RF and TSS since specific combinations of GAS and hosts appear to be necessary for the disease to occur.

The present report describes bacterial and host determinants of non-related Belgian toddlers who developped RF after TSS. A molecular analysis of both the bacterial emm-type and superantigens (exotoxins that are phage-encoded) and HLA host factors was carried out in an attempt to decipher the combination that may lead to such uncommon, but very similar presentations.

Methods: The diagnosis of TSS and RF were based on the consensus definition and modified Jones criteria. emm-typing was performed using the CDC sequencing protocol. The presence of 17 superantigen genes was detected by PCR and sequencing. Phages lysates were obtained by mitomycin C induction and observed by electron microscopy. The HLA molecular typing was performed using PCR and sequence-specific oligonucleotide genotyping method.

Results: A 13-month-old boy and a 14-month-old girl presented a GAS TSS with fever, severe hypotension, renal impairment, coagulopathy, thrombocytopenia and meningitis. They were treated with penicillin, clindamycin, intravenous immunoglobulin and intensive care supportive therapy. After 2 and 3 weeks respectively, multiple subcutaneous nodules as well as migratory polyarthritis or monoarthritis developed in both children. RF diagnosis was established since 1 and 2 major plus 3 minor Jones criteria were fulfilled for the 2 children respectively. The molecular analysis of bacterial virulence genes revealed that the 2 GAS isolates belonged to the usual, although distinct, invasive emm-types 1

and 3. Both isolates carried a wide set of prophage-encoded virulence factors, with only the speG and speA superantigen-encoding genes in common. Both patients shared the HLA DQB1*0301 allele which has been associated with susceptibility to GAS necrotising fasciitis.



Morphological diversity bacteriophages in GAS isolates 1 and 2. Transmission electron microscopy after negative staining shows 4 bacteriophages belonging to the siphoviridae family. They present icosahedral heads of variable size and morphology (B and C: 45 nm, A and D: 55 nm) and non-contractile tails of variable length (A: 230 nm, B: 240 nm, C and D: 180 nm) (Magnification ×160,000).

Conclusion: Our study exemplified the complexity and modularity of the host-pathogen interactions in GAS diseases. More documented reports and systematic studies are needed to better understand the underlying molecular and physiologic mechanisms involved in such severe pathologies.

P2225 Effectiveness and safety of short versus long duration of antibiotic therapy for group A streptococcal tonsillopharyngitis: a meta-analysis of randomised controlled trials

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Objective: Compliance to traditionally recommended 10-day penicillin treatment for acute group-A streptococcal (GAS) tonsillopharyngitis is rather limited. To evaluate the effectiveness and safety of short-course antibiotic treatment for streptococcal tonsillopharyngitis, we performed a meta-analysis of randomised controlled trials (RCTs).

Methods: We searched in PubMed, the Cochrane Central Register of Controlled Trials, and bibliographies of relevant articles, to identify RCTs performed on patients of all ages, with GAS tonsillopharyngitis, verified by microbiologic or serologic methods, comparing short-course (7 days or less) versus long-course treatment (at least 2 days longer than short-course), with the same antibiotic, in the same total daily dose.

Results: Eleven RCTs (6 open-label), performed on children and adults, with GAS tonsillopharyngitis (verified microbiologically in 10 RCTs, and microbiologically or serologically in one RCT), comparing short-course [orally administered penicillin V (3–7 days), cephalosporins (5–7 days), clindamycin (5 days), or intramuscularly administered ceftriaxone (1 day) in 5, 4, 1, and 1 RCTs, respectively] versus long-course treatment (7–10 days for oral or 3 days for intramuscular agents), were included. Microbiological eradication rates of GAS were inferior for short-course compared to long-course treated patients included in all RCTs [11 RCTs, 1887 patients, fixed effect model (FEM), odds ratio (OR)=0.42, 95% confidence interval (CI)=0.31–0.56]; RCTs involving mainly children (8 RCTs, 1372 patients, FEM, OR = 0.55, 95% CI=0.38–0.78); RCTs comparing 5 to 10-day therapy (6 RCTs, 1167 patients, FEM, OR = 0.51, 95% CI=0.34–0.76); RCTs comparing penicillin V regimens (5 RCTs, 720 patients, FEM, OR = 0.35, 95% CI=0.24–0.51). Findings were marginally non-significant for patients treated with cephalosporins (5 RCTs, 1078 patients, FEM, OR = 0.61, 95% CI=0.37–1.01). Additionally, for patients that received short-course compared to long-course treatment, clinical success was inferior (6 RCTs, 1277 patients, FEM, OR = 0.46, 95% CI=0.28–0.75), short-term relapses were not different, long-term recurrences were more frequent, and adverse events were not different.

Conclusion: The findings of this meta-analysis reinforce the idea that acute tonsillopharyngitis due to GAS should be treated for the traditionally recommended duration of 10 days, in order to maximize microbiological eradication, and achieve superior clinical outcomes.

P2226 Evolution of the incidence of early-onset and late-onset group B Streptococcal disease before and after prevention policies – Barcelona 1994–2007

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Objective: Between 1994 and 1999 the prophylactic strategy based in universal prenatal group B Streptococcal (GBS) screening and intrapartum antibiotic for GBS-colonized women, and for women with unknown status and delivery <37 weeks, amniotic membrane rupture or intrapartum fewer, was progressively implemented in 10 Barcelona-area hospitals. The aim of the study is to continue monitoring trends of early-onset GBS disease in these 10 hospitals, and to assess if the observed decreases in its incidence has been accompanied by increases in the incidence of late-onset GBS disease.

Results: From 1994 to 2006, 282.046 live infants were born in our hospitals. Early-onset GBS disease, defined as the presence of GBS in a sterile fluid (blood, CSF, etc) within the first week of life, was diagnosed in 219 neonates. During the 12 years of the study the incidence decreased as follows: 2.25 per 1000 in 1994, 1.88 in 1995, 1.53 in 1996, 1.23 in 1997, 0.66 in 1998, 0.48 in 1999, 0.56 in 2000, 0.39 in 2001, 0.47 in 2002, 0.41 in 2003, 0.24 in 2004, 0.58 in 2005 and 0.23 in 2006 These rates indicated that over this period of time the incidence decreased by 83% (RR 0.830; 95% CI 0.799–0.863, P < 0.001). In the year 2006, incidences in individual hospitals ranged from 0.54 to 0 per 1000.

From 1996 to 2006, 245.672 live infants were born in our hospitals. Late-onset GBS disease, defined as the presence of GBS in a sterile fluid (blood, CSF, etc) in infants aged between 8 and 90 days of life, was diagnosed in 90 cases. During the 10 years of the study the incidence fluctuated as follows: 0.11 per 1000 in 1996, 0.51 in 1997, 0.36 in 1998, 0.39 in 1999, 0.14 in 2000, 0.22 in 2001, 0.35 in 2002, 0.25 in 2003, 0.60 in 2004, 0.58 in 2005 and 0.42 in 2006 (RR 1.068; 95% CI 0.998–1.143, P =0.057). Although not significant, these rates of incidence suggested a slightly tendency to increase annually by 6.5%.

Conclusions: The application of prevention measures supposed a substantial decrease in the incidence of early-onset GBS disease during the firsts years and a maintained low rates till now. In contrast, the incidence of late-onset GBS disease fluctuated over the years, and although not significant, there it seems to be a slightly tendency to increase, suggesting that intrapartum antibiotics is not effective against late-onset GBS disease. Continue surveillance of late-onset GBS disease is needed to monitor the impact of the recommended prophylactic strategy.

P2227 **Evaluation of a real-time PCR (GeneXperTSystem) for the diagnosis of intrapartum group B *Streptococcus* colonisation**

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Streptococcus agalactiae (GBS) is the leading cause of neonatal sepsis and meningitis. Maternal screening for GBS colonisation is critical for providing an appropriate prophylaxis and avoiding early-onset neonatal GBS disease. The antenatal screening of pregnant women from immigrant population is not appropriate in some communities; so unknown intrapartum colonisation status is increasingly frequent in this group.

Objectives: To compare prospectively the performance characteristics of a rapid molecular technique, GeneXpertSystem (Cepheid) versus conventional culture for intrapartum SGB detection. To describe the clinical impact of rapid delivery results.

Methods: A vaginal and rectal sample was obtained during labor for conventional culture and RealTime PCR, during a six month period, following these criteria: unknown colonisation status or colonisation status older than 5 weeks before partum. Each swab sample was processed by culture in selective media. A PBS buffer was used to recover the sample from the collector. It was centrifuged and 200 mcL of the suspension were inoculated on the detection cartridge obtaining the results in 90 minutes. Clinical record for every pregnant woman was followed up before and after providing GBS colonisation results

Results: Forty-four samples were collected. Overall intrapartum colonisation rate was 22.7%. Conventional culture was positive in 7 women (15.9%) and RealTime PCR in 10 (22.7%). Antibiotic prophylaxis was administrated in 8 of 10 women when RealTime PCR was positive. Only 2 of 34 (5.8%) women with negative result for RealTime PCR were treated following different clinical criteria but SGB colonisation.

Conclusions:

1. GeneXpertSystem is more sensitive than conventional culture for detecting SGB in pregnant women.
2. A negative result for RealTime PCR was conclusive in order to avoid antibiotic prophylaxis in 94.1% of the negative cases.
3. Use of GeneXpertSystem during labor may lead to a potencial reduction of neonatal infection screening tests and avoid days of neonatal sepsis treatment.

Internet and electronic resources

P2228 **Improving the quality of care for community-acquired pneumonia using Web-based support for dissemination, communication and measurement**

P.G. Davey, S. Leonard, A. Patton, C. Bucknall on behalf of the Scottish National Audit Project on Community Acquired Pnemonia

Objectives: Our objective is to improve management of CAP by defining and implementing a bundle of essential elements of care that must be delivered within the first few hours after arrival at the hospital.

Methods: This prospective improvement study is part of a Scotland National Audit Project funded by the Health Foundation and co-ordinated by the Royal Colleges of Physicians in Scotland (www.snaproject.org.uk/). We established a multi-disciplinary Steering Group to develop a care bundle that identifies critical care processes in the first 4 hours after presentation. Care Bundles focus on clinical actions that are proven (with high level evidence) to improve outcomes. We are testing bundle implementation and measures for improvement in six hospitals before spreading to hospitals in all 15 Health Boards across Scotland. We are using rapid cycle tests of change and small, repeated measures to bring about change and measure impact. We have established a password protected Extranet using the service provided by the Institute for Healthcare Improvement (www.ihi.org). The Extranet provides easy access to all of the documentation for the project, aids communication between teams and allows teams to view their results in the context of aggregated data from all participating hospitals. The cost of an Extranet is $6,000 per year and requires 4–5 working days to establish.

Results: All six hospitals have posted measures on the Extranet although the number contributing measures each month has ranged from three to six. Overall the results show progressive improvement in individual care processes (for example from 17% to 65% for CURB65 risk assessment) and from 4% to 32% for compliance with all bundle elements (⩾92% oxygenation and antibiotics within 4 h plus identification of patients requiring a high dependency plan and of low risk patients who can be managed at home. Individual hospitals have used a variety of techniques to drive these improvements (e.g. email of monthly results to junior doctors, wall posters, reminders on case records).

Conclusions: An Extranet is a practical solution for web based support for quality improvement projects. Although steady progress has been achieved with improvement in the quality of care for CAP the overall compliance with the full care bundle is currently well below our target level of 95%. The main barrier that remains to be overcome is sustaining improvement despite increasingly frequent changes of junior medical staff.

P2229 **PAUSE: Prudent Antibiotic User. A collaborative web-based forum for sharing experience and learning resources between providers of education about prudent antibiotic prescribing**

P.G. Davey, D.N. Nathwani, I. Gyssens, I. Harding, M. Akova (Dundee, UK; Nijmegen, NL; Upwell, UK; Ankara, TR)

Aim and objectives: The aim of PAUSE is to ensure that practitioners will be prudent prescribers of antibiotics and will promote prudent use of antibiotics in whatever clinical context they are working in. Objectives are to:

- Construct a framework of learning outcomes that defines a prudent prescriber.
- Provide flexible web-based learning resources and assessments that will enable undergraduate medical students to achieve clearly defined learning outcomes in prudent prescribing, for their level of expertise.
- Provide an exemplary learning platform in prudent antibiotic prescribing for the development of other healthcare professionals.

Background: In 2003 the Scottish Executive funded the University of Dundee and the British Society for Antimicrobial Chemotherapy (BSAC) to develop a web based learning resource for undergraduate medical education. The site was launched in February 2005 and has been included as a core component of the University of Dundee Medical School curriculum since August 2005. In 2006 BSAC began a further 3 year development to transfer the resources to a new website (PAUSE) with improved functionality and extension of the content to meet the needs of different professional groups. In 2007 ESCMID and the ESCMID Study Group for Antibiotic Policies (ESGAP) agreed to partner BSAC in the development of the site.

Results: The PAUSE website was launched in August 2007: http://www.pause-online.org.uk/ By June 2008 160 medical students at the University of Dundee will have used the materials. Examples of feedback from students include: “I learnt more about antibiotics in that week than I had in the previous two years”; “I really felt that we were learning practical things that will be used regularly when I am a qualified doctor”; “Studying the web-based vignettes was useful as it provided cases to work through which makes teaching more memorable”; “The web-based material was excellent. This was a real help and can be used to refer to at a later date”; “The short cases and patient report gave structure to the week”. NHS Education Scotland have used resources from the PAUSE site to develop e-learning and assessment modules in the Doctors’ Online Training Scheme, a compulsory course for doctors in the first two years after qualification in Scotland.

Conclusions: The PAUSE website is an established resource that can be expanded through development and partnership between BSAC, ESGAP, ESCMID and its affiliated Societies.

P2230 Integrated international molecular databases in monitoring the emergence and spread of infection

T.J. Dallman, J. Green, S. Gnaneshan, R. Myers and the DIPNET, EUROROTA and HepSEQ steering committees

Objectives: Integrated molecular databases associated with Dedicated (disease specific) Surveillance Networks (DSNs) can fulfil a pivotal role in tracking the spread of infectious diseases. The Health Protection Agency hosts a wide range of molecular typing databases as components of National or International Surveillance Networks. Here we describe three infectious disease resources from the international DSNs associated with; diphtheria (DIPNET), rotavirus (EUROROTA) and hepatitis B (HepSEQ) and identify key elements in successfully establishing and implementing them.
Methods: On-line, integrated databases were established providing a central portal for the submission and integration of disparate data types. These included clinical data (e.g. serology), demographic data (e.g. country of infection, sex, age), epidemiological data (e.g. transmission route, outbreaks) and molecular data (e.g. nucleotide and protein sequences, mutation information). On-line databases allowed the storage of this data in a robust, highly structured way that can be updated, accessed and queried from any location in real time. A number of technical and non-technical issues impact on the success of these resources. Through the projects described we have analysed and identified the key elements impacting on the successful development of an online resource.
Results: The foundation of integrated online DSNs, as well as facilitating the amalgamation of disparate data types also requires the development of computational tools to query and display the data. DIPNET and HeqSEQ provide the ability for users to determine the genotype of their isolate through the automated interrogation of ribotype profiles and nucleotide sequences respectively. EUROROTA enables the construction of graphical representations of integrated epidemiological and molecular data further underpinned by demographic data.
Conclusions: Global approaches including the establishment of international laboratory-based surveillance networks are imperative for the control of emerging and established infectious diseases. A network of reference microbiological laboratories with similar technological capabilities and molecular typing methods are required to allow the coordination of surveillance activities and the standardisation of methodologies and protocols. Efficient disease surveillance requires the collection and integration of disparate data types which can then be utilised to provide enhanced disease surveillance.

P2231 e-meducation.org: an open access medical education web portal focused on infectious diseases

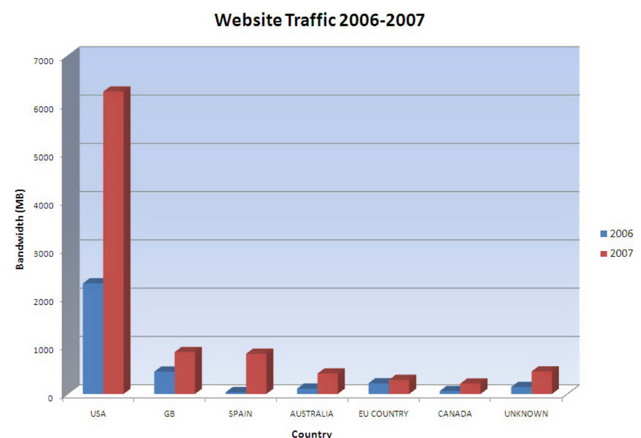
V. Alexiou, M. Falagas (Athens, GR)

Objective: Internet can serve in opening the door to a brand new world of high quality medical information. However, the chaotic size of data available in the WWW is often misleading. We sought to provide the world medical community with a web portal that may be used as a clearinghouse providing the outlet for dissemination of high quality WWW educational products, currently focused on infectious diseases.
Methods: Directories of open access WWW resources have been compiled and others are being currently under development in the field of infectious diseases. Future development aims to cover most medical fields. A custom-built medical search engine was created. Really Simple Syndication (RSS) feeds and video sharing services were reviewed for their quality and were presented along with case-based educational presentations through a user-friendly web portal interface. A directory of guidelines database is also currently under development.
Results: The educational portal "e-meducation" available at http://www.e-meducation.org/ has been launched in December 2006 and at the moment, provides links to more than 1000 educational web-pages, more than 2100 clinical practice guidelines, 32 news feeds, and 14 educational videos. The web site also hosts 40 case-based presentations (almost all from the field of clinical microbiology/infectious diseases) and a custom medical search engine. Within a year e-meducation.org has received more than 142000 visits.
Conclusion: Based on the incorporation of simple and tested educational strategies such as case based instruction and interactive learning, e-meducation.org aims to become a prototype platform that offers a more convenient interface to existing products, resources and medical contents.

P2232 MicrobLog: an online information resource for those studying or training in medical microbiology, virology and infectious diseases

A. Hussain (Leicester, UK)

The World Wide Web (WWW) is an abundant source of learning for all health professionals. The range of learning tools are widely varied from free websites to those that are subscription based. The Royal College of Pathologists (RCPath) in the United Kingdom (UK) issues the curriculum and sets the examinations for gaining membership. Although trainees are made aware of online resources, there are very few resources aimed specifically at trainees in medical microbiology and virology. The RCPath is currently undertaking a review of electronic learning resources.
There is a paucity of readily accessible information specific to the needs of a trainee microbiologist in the UK. Whilst there is a very good resource in the UK for trainee virologists, no such website exists for microbiologists and infectious disease physicians.
I found that recording anecdotes and my personal experience became invaluable as a learning tool. In preparation for professional examinations, the experiences of trainees are varied across the country and an appropriate forum for discussion was not available. The MicrobLog website (http://microblog.me.uk) was started in May 2006, as a result of my concerns. The aim of the website is to bring these disciplines together, primarily with an emphasis on medical microbiology. The forum section allows trainees to discuss local, national and international training opportunities as well as specific areas of research and development.
Since July 2006, we have had 730 692 hits, 25.6% of which were unique visitors. 37.7% of all hits originated from the USA, with only 4.6% originating from the UK. The commonest searched phrases were "taurolock", "acinetobacter" and "ganciclovir", with the commonest referrer being www.google.com. Currently the site receives 400 hits per day, 24.8% of which are unique visitations. The worldwide distribution of website traffic is shown in Figure 1.



The website usage data has highlighted an interesting point. There are many North American microbiology e-learning websites, covering both the practical and the theoretical. These websites contain factual as well as online assessment tools, but their content is not always relevant to practicing in the UK. Although medical microbiology and virology is a small speciality, there is a significant need to expand the number

of internet sites with validated content and assessment tools, to bring medical microbiology and virology in line with other disciplines.

P2233 www.ccrbtyping.net – a web-based typing tool for staphylococci

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Objectives: ccrB typing, based on the DNA sequencing of an internal fragment of ccrB, was developed as a potential first-line SCCmec typing strategy for meticillin resistant *Staphylococcus aureus*, since the ccrB sequence is part of the ccrAB locus, whose allotypes are used for the definition of SCCmec types. Clustering of ccrB sequences has been shown to properly discriminate between different SCCmec types (I, II, III, IV and VI) (Oliveira et al, J. Antimicrob Chemother 58:23–30). To make this approach available to the general public, a web based typing tool interface was developed so that users can submit ccrB sequences and obtain an automatic assignment to a ccrB allele and presumptive SCCmec type classification.
Methods: The website serves as a front-end for a database and user management system, coupled with two sequence alignment programmes (MUSCLE and Clustal) and an alignment and tree viewer software (Jalview). For each SCCmec type, a set of known sequences are defined as a prototypes. The user submitted ccrB sequences are then, after an automatic trimming, compared by multiple sequence alignment with the defined prototypes, and the most similar prototype defines the ccrB allele, allowing the inference of ccrAB allotype and SCCmec type.
Results: The website (www.ccrbtyping.net) is being used since late October 2007. At the website launch date the database had 98 ccrB sequences, including 17 prototype sequences, with references and with other important isolate information. All the public information can be easily extracted to csv format and an online tutorial explains the users how to work with the web tool. The data can be saved privately or it can be submitted to the public database after being reviewed by a system curator.
Conclusion: Web-based applications have proved to be the best approach for the comparison of sequence based typing methodologies results. Anyone with internet connection can contribute with their data to the public database and help to validate and improve the typing methodology. The implemented website has demonstrated its usefulness for ccrB typing giving good results for several staphylococci species, allowing for an expanding database of curated ccrB sequences. Additionally the web based typing tool interface was implemented to be rapidly configurable, and can be used as a framework for other sequence based typing methods based either on direct sequence similarity or on the comparison of repeated patterns.

P2234 Development of a web-based learning tool to enhance healthcare workers′ knowledge, attitude, and risk perception about safe work practices concerning meticillin-resistant *Staphylococcus aureus*

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Objectives: To develop a web-based learning tool based on the available national Meticillin Resistant *Staphylococcus aureus* (MRSA) policy, to enhance healthcare workers' (HCWs) knowledge, attitude, and risk perception concerning safe work practices.
Methods: A validated questionnaire and scenario-based tests were used to determine HCWs' key questions about safe work practices regarding MRSA. Next, the questions were categorised into groups by the Card Sort Method and analysed by WebSort software in order to provide a user-centred search structure for the web-based learning tool. The study was conducted among physicians, nurses, and domestic staff in four hospitals in Germany and the Netherlands.
Results: The results of 276 questionnaires showed inadequate levels of knowledge (97% of respondents) and risk perception (24%) about safe work practices, and negative attitudes (63%) toward complying with these practices. The 28 additional interviews demonstrated that lack of knowledge is mainly related to being uninformed about transmission routes of MRSA (14%), resulting in an inadequate perception of the personal risk to obtain and transmit MRSA (10%). A negative attitude is caused by HCWs questioning the usefulness of adhering to safe work practices (14%). These factors lead to non-compliance with safe work practices. HCWs seem to favour a more personal and social approach of safe work practice documents, stressing personal risks and the rationale behind applying the practices. In sum, 167 key questions were found about which the knowledge, the attitudes or the risk perceptions were unsatisfactory. The web-based learning tool should address these questions. The Card Sort Method (n = 10) resulted in ten categories in which the key questions can be grouped. HCWs are best served with a web-based system in which they can actively search these questions in their own language both via a search engine and a table of contents representing HCWs' terminology and information needs.
Conclusions: Our study indicated that the mixed-methods design provides a powerful approach to analyse HCWs' key questions concerning safe work practices, and provides us with a search structure for a user-centred web-based learning tool. HCWs' involvement in the development of the tool might encourage compliance with safe work practices, which remains subject for further investigation.

P2235 National software system for microbiology laboratory management in Bulgaria: "Clinical Microbiology and Nosocomial Infections"

M.M. Petrov, B. Kojuharov, T. Velinov, T. Kantardjiev (Sofia, BG)

Expedient software systems in clinical microbiology laboratories constitute very useful and effective component in laboratory and hospital management. Susceptibility test results of dozens of thousands of laboratories around the world are stored in paper or in computer files, which makes them inaccessible for analysis and comparison. The "Clinical Microbiology and Nosocomial Infections" (CMNI) programme provides a software through which data from each laboratory are entered into a common code and file format for the corresponding laboratory. These data could be shared with another laboratory working on CMNI programme for further collaboration, and utilised, through the main server, based in the National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria, for a national data analysis and implementation of various control measures.
CMNI programme is web-based database software in Bulgarian language for the management of microbiology laboratory results and global surveillance of bacterial resistance to antimicrobial agents and control of nosocomial infections. Significant parts of HELICS protocols are implemented in the programme and CMNI software is also compatible to, and capable to interface with other Laboratory Information Systems – LIS (e.g. WHONET), the Antibiotic Consumption Calculator (ABC Calc), and other statistical packages and programmes.
The CMNI programme comprises the following three principal components:

- Laboratory configuration;
- Data entry;
- Data analysis

The analysis of the information facilitates:

1. The studying and understanding of trends of resistance;
2. The detection of epidemics and nosocomial infections;
3. The differentiation of epidemic from endemic infections;
4. The tracking and evaluation of financial expenditures in the laboratory;
5. The assessment of various risk factors;
6. The monitoring of antibiotic consumption;
7. The development of national antibiotic policy.

The ongoing equipment with LIS "CMNI" is a key component of the local and national surveillance of antimicrobial resistance,

antibiotic consumption and nosocomial infections' control in Bulgaria. Bulgarian Surveillance Tracking of Antimicrobial Resistance (BulSTAR) along with the National Laboratory Management System for clinical microbiology in Bulgaria "Clinical Microbiology and Nosocomial Infections" constitute the basic components of the Bulgarian national strategy, aimed at antimicrobial resistance control and prevention and prudent use of antimicrobial agents.

P2236 Modern information technologies in physicians' education in the field of socially most important diseases in Russia and beyond

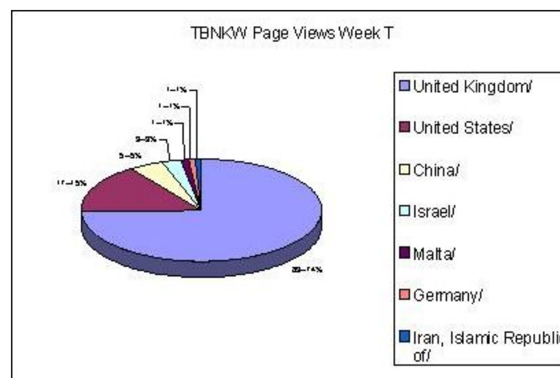
O. Stetsiouk, Y. Belkova, V. Kostin (Smolensk, RU)

Objectives: Traditional forms of post-graduate education do not completely meet needs of professional development of physicians. It is especially evident in countries with large territories and high prevalence of socially most important diseases (SMID). Therefore distance education (DE) is of a priority setting and perspective form of improving physicians' professional level. The aim is to summarize experience in development of distance education (DE) on antimicrobial therapy (AT) of SMID in Russia and beyond as a possible way of post-graduate education for healthcare professionals in such important topic.
Methods: The educational course "AT of socially most important diseases" was implemented in Internet centre of DE on the basis of the web-site "Antibiotics and Antimicrobial Therapy" (www.antibiotic.ru) founded under the auspices of the Institute of Antimicrobial Chemotherapy (IAC), the Department of Clinical Pharmacology of Smolensk State Medical Academy with the support of the United States Pharmacopeia (USP) and the United States Agency for International Development (USAID).
Results: The course "AT of socially most important diseases" consists of 14 specific topics covering diagnostics, treatment and prophylaxis of HIV-infection, tuberculosis, chronic viral hepatitis and STDs. Every topic comes complete with educational materials and on-line tests. Upon completion of each topic "students" are offered control tests to enable tutors evaluation of their level of knowledge. The final exam is held in the presence of tutors and includes testing and interview. After passing final exam "students" are awarded the official certificates.
Since 2004, more then 200 physicians from 26 Russian regions, Belarus, Kazakhstan, Kyrgyzstan, Lithuania and Ukraine have been trained in SMID.
IAC provides the support to satellite DE centres in training of their tutor staff and developing a system for independent work. Currently 11 regional DE centres in Ekaterinburg, Novosibirsk, Penza, Samara, Vladivostok (2 centres), Minsk, Vitebsk (Belarus), Dnepropetrovsk (Ukraine), Osh (Kyrgyzstan) are working.
Conclusions: 1) DE via Internet is upcoming form of physicians' education in SMID. 2) Advantages make DE convenient, effective and demanded. 3) DE extension with regional centres is promising for further development.

P2237 What is the impact of a Tuberculosis National Knowledge Week?

F. Hansraj, A. Roy, G. Jawaheer, P. Kostkova, J. Freed, M. Catchpole, S. Wiseman (London, UK)

Objectives: National Knowledge Weeks (NKWs) highlight the best current evidence, enabling busy healthcare professionals to quickly and easily find relevant and reliable information. Our aim was to evaluate the impact of an NKW on the dissemination of tuberculosis (TB) resources.
Methods: The National electronic Library of Infection (NeLI) www.neli.org.uk collaborated with the Health Protection Agency www.hpa.org.uk to host a Tuberculosis National Knowledge Week http://www.neli.org.uk/IntegratedCRD.nsf/TBNKW?OpenForm from 26th to 30th March 2007, which was timed to link in with World TB day on 24th March.
NeLI is of the Specialist Libraries of the National Library for Health www.library.nhs.uk. It is a single-entry portal to evidence-based medical knowledge around infection, providing access to the best available evidence on prevention, treatment, investigation and control, with resources that are quality appraised by healthcare professionals. This makes it ideal to disseminate new TB resources.
This NKW comprised of a comprehensive collection of recent, peer-reviewed publications on TB. In addition, experts in the fields of TB epidemiology, management and treatment provided short reflections. A flyer to promote the TB NKW and the corresponding resources hosted on NeLI was sent to 512 NHS libraries across England and these were displayed before and during the NKW.
Results and Conclusions: Web server logs were used to analyse the impact of this NKW, taking into account the number of page views received, the popularity of the page, the geographical location of the incoming traffic, the means of accessing the page and the outgoing traffic from the page. The impact of this NKW on overall NeLI traffic was also analysed.
Analyses found that prior to, during, and a week after the NKW, the NKW page was the 3rd most frequently visited on NeLI (3.31%). Figure 1 shows the geographical distribution of users of the page during the TB NKW.



The impact of the NKW on overall NeLI traffic was also significant; there was an increase in the number of page views to NeLI in the two weeks prior to the NKW, which reached a peak during the NKW itself. However, merely hosting the NKW page could not account for this rise in page views. Therefore, it is likely that the flyers distributed prior to the event also had an influence.

P2238 "DelioCode": a freeware programme for comparing oligonucleotidic sequences by verification codes

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PCR is a very diffused technique to amplify nucleic sequences by means of specific primers, that are continuously developed and published on international journals. Sometimes transcription mistakes can occur and erroneous primer sequences can be published, resulting in unexpected results if experiments are replicated. Although primers are relatively short, a rapid system for checking the correctness of the transcribed sequences could mitigate this problem. Verification codes are already used in other disciplines to guarantee the correctness of codified information. In this work an algorithm has been defined and the related software tool developed to fill this gap.
A generic algorithm based on the ASCII coding of characters and their position within the sequence has been defined to generate a specific code on the basis of the sequence composition. Configuration parameters such as amount of characters and coding syntax have been left open for modification and personalisation. Microsoft Visual Studio 2005 has been used to compile "DelioCode" (Descriptive Literal Oligonucleotides Code), a Windows application featuring the algorithm and available on the web for download. The default algorithm has been used to verify the correctness of several sequences by testing casual primers with induced mistakes and primers published sequentially by different authors.

All induced mistakes were detected by comparing the verification codes. On the basis of DelioCode, some typos have been found in primers sequences published in peer reviewed journals, and PCR repetition with these wrong primer sequences did not result in DNA amplification.
The DelioCode programme is a tool for rapidly generating a unique code on the basis of a specific sequence. The shortness of the performed verification codes allows a rapid comparison among the sequences to find the common mistakes. This is useful every time researchers need to check the correctness of specific sequences, such as when sequences are transcribed from published articles or when oligonucleotidic primers are orderable by compilation of on-line forms.
The diffusion of the verification codes generated by the programme would avoid typing mistakes in the transcription and would allow to save time in old-fashioned manual double-checking. The generation of specific codes for every primer could be evaluated to create a freeware "verified primer sequences bank", where scientists could find rapidly specific primers already tested and published.

P2239 10 years of the *Aspergillus* Website (www.aspergillus.org.uk)

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Objectives: Information on *Aspergillus* and aspergillosis has been widely distributed and often difficult to access. The *Aspergillus* website launched in 1998 with the primary objective of providing an easily accessible encyclopaedic resource on all Aspergilli, their biology, genomics and diseases that they cause.
Methods: The evolution and expansion of the website over its first 10 years of operation were reviewed, including site statistics.
Results: The website was aimed at the professional reader and offered help and advice on medical and scientific issues relating to *Aspergillus*. Quality of information is paramount.
Content covered a range of topics including diagnosis and treatment for medical readers. As *Aspergillus* genomics grew, so the section expanded. Sections on veterinary and air quality were added. Historical articles and summaries were important additions. The library of articles has grown to provide 1232 pdf's. More recently sections providing educational materials (slides & video) and complete case histories have been added. Content specifically for research scientists includes a research directory, laboratory protocols and genomics pages, species (n = 847 names) and secondary metabolite (n = 354) databases and numerous links. There is a catalogue with links to all mycology societies. Upcoming conferences are organised by date with links and we hold over 6000 full abstracts from many past conferences, and many downloadable full colour posters. More recently sections on secondary metabolites and species names have been added, expanded and integrated. A distinctive feature are the hundreds of images made available for non-profit and commercial use.
The *Aspergillus* website newsfeeds were initiated 18 months ago and currently provide 20,000 feeds per month.
On the 10th anniversary of the website, we have >35,000 registered members, a patient support group of >500 highly active members and

Figure 1. *Aspergillus* Website Statistics 2003 – 2007

we provide 3 million website pages a year. >25,000 distinct users access the website each month – ~850 different people per day (Figure 1). 1800 websites link to the *Aspergillus* website.
Conclusions: The *Aspergillus* website is a premier resource for those interested in aspergillosis and the Aspergilli.

P2240 *Aspergillus* for Patients (www.aspergillus.org.uk/patients)

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Objectives: Information and support on *Aspergillus* illnesses is difficult to obtain for patients and carers. The *Aspergillus* for Patients section of the *Aspergillus* website was intended to make information on *Aspergillus* and Aspergillosis easy to access and understand for the layperson.
Methods: A short history review of the current *Aspergillus* for Patients website, including site statistics.
Results: We set up the first *Aspergillus* support group as a simple mailing list to coordinate questions and answers from patients but the group rapidly outgrew that arrangement and we moved it to a fully featured webpage/email discussion group in January 2003. The *Aspergillus* Support group (www.yahoo.co.uk/AspergillusSupport/) now has 527 members and still growing steadily.
A lot of information was requested repeatedly so a small website (The Patients website) was set up to provide consistent answers to the common questions. These pages remain one of the most popular sections of the *Aspergillus* website though some of its functions have been taken over by the *Aspergillus* Trust's website (www.aspergillustrust.org) with which it remains closely allied – the combined viewing figures are 25,000 page requests per month.
The *Aspergillus* Trust charity arose directly from the Yahoo Support group and is governed by some members of that group in close collaboration with the *Aspergillus* website.
The Patients website collected information direct from patients to try to get a better idea of the range of illnesses people were seeking information on. 1,232 patients filled out the form over the next year or two and the information gathered broke down to the following numbers; Invasive aspergillosis – 249, CPA – 142, Aspergilloma – 134, ABPA – 359, Allergies – 317, Sinusitis – 355, SBS (Sick building syndrome) – 167, Other – 292. Substantial numbers of rare illnesses e.g. ABPA and aspergilloma are represented well in this group, indicating we are reaching our target audience effectively.
Conclusions: The Support groups and *Aspergillus* for Patients website section is a major resource for information for the layperson on these illnesses.

P2241 Publication rate of Turkish infectious diseases and clinical microbiology and microbiology and clinical microbiology specialisation theses and microbiology doctorate theses, in international peer-review journals

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Objectives: To investigate the publication rate of Turkish infectious diseases and clinical microbiology (ID&CM), microbiology and clinical microbiology (M&CM) specialisation theses and microbiology doctorate theses in international peer-review journals.
Methods: On 17 August 2007 the thesis database of the Council of Higher Education of the Republic of Turkey where all specialisation and doctorate theses are recorded obligatorily, was searched for ID&CM and M&CM specialisation theses and microbiology doctorate theses. The publication rate of these theses was found out by searching Science Citation Index-Expanded database for thesis author and mentor between 17 August–12 September 2007. For statistical comparisons Chi-square test was used and a p value less than 0.05 was considered as significant.
Results: Our search yielded a total of 834 theses (268 ID&CM speialisation theses, 312 M&CM specialisation theses, 254 doctorate theses). It was found that the overall publication rate was 11.2% (94/834). The rates for doctorate, ID&CM and M&CM specialisation theses

was 13.3% (34/254), 10.0% (27/268), 10.5% (33/312), respectively ($p > 0.005$). The publication rate of 2002–2007 theses was 9.3%, whereas 1997–2001 theses was 13.1% ($p = 0.08$). It was determined that nine of the 94 published theses were theses of 1997–2001 period and 85 of 2002–2007 period.
Conclusion: Despite an increase in 2002–2007 period (Probably due to updated criteria of the Council of Higher Education of the Republic of Turkey for academic promotion in 2000), publication rate of the investigated theses in international peer-review journals is very inadequate. Thesis is an important part of specialisation and doctorate education and necessitates intense work. The created knowledge usually contain important data about the country and the world. Publication of the theses supply dissemination of new knowledge and completes the process of scientific study. Solutions must be generated to promote the publication of specialisation and doctorate theses.

P2242 Designing online, educational games about microbes, hand and respiratory hygiene and prudent antibiotics use for junior pupils across Europe

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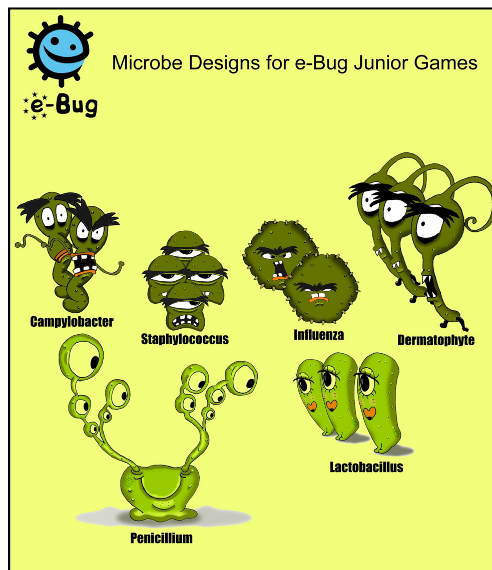
Objectives: e-Bug is a European-wide, EC funded, antibiotic and hygiene teaching resource. It reinforces an awareness of microbes, hygiene and prudent antibiotics use. Our objective is to create web-based games for school pupils in 17 European countries that complement the e-Bug educational pack. The pack and games fulfill and extend school curricula. As games will be available for use during school and pupils' own time, they cannot require that the player has been taught the subject. They also need to be enjoyable enough for pupils to play by choice.
Methods: To teach appropriate placement of food in a fridge, we designed a sorting game where the player competes against a clock to store shopping. Other outcomes were taught through the metaphor of being shrunken inside the human body in the style of a platform game, such as Super Mario Brothers, where the player interacts with good and bad microbes and medicine.



Design Evaluation and Results: Prior to learning outcomes being agreed, game ideas were discussed in a series of focus groups with 10 school teachers in the UK.Unlike many 'edutainment' games which are themed appropriately but which separate playing from teaching, we designed the e-Bug games to use 'stealth learning' techniques where the games incorporate learning outcomes into their core design in a fashion that allows pupils to be taught realistic science through the act of playing. Evaluation questionnaires were given to children and used to decide which artistic look was appropriate.A workshop was carried out with teachers and partners from each of our partner countries where we discussed potential approaches to the games and defined our learning outcomes. At this stage, ideas were discarded that would not appeal to junior pupils or reinforce e-Bug's messages. Designs based around the final learning outcomes were iteratively circulated to teachers and updated based on their feedback.
Conclusions: Early versions of the games will be playable in January 2008. These will be focus-tested with pupils in the UK and updated prior to an initial launch in February. The pack is being evaluated from January through December 2008 in a total of 45 junior schools in France, the Czech Republic and the UK.We will draw from these schools to evaluate the effectiveness and usability of the website and games. Web log analysis and observational studies will be conducted in the UK, Czech Republic and France to determine knowledge gained, usability and level of enjoyment by pupils.