Progress in pharmacokinetics and pharmacodynamics - I

P1022 Pharmacokinetics of telithromycin in plasma and soft tissue after single-dose administration in healthy volunteers

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Objectives: Telithromycin was described to reach high concentrations levels in inflammatory fluid, in bronchopulmonary tissues and in tonsillar tissue. Because of these data telithromycin is speculated to be a new option in the therapy of skin and soft tissue infections. To determine the concentration of telithromycin in the interstitial space fluid, the pharmacokinetics of this new antibiotic were assessed after single dose administration in young healthy volunteers by the use of microdialysis.
Methods: Plasma- and extracellular samples (subcutaneous adipose tissue a. skeletal muscle) were taken from 10 healthy male volunteers by a venous catheter (plasma) and by microdialysis (soft tissue) every 20 min over a period of 8 h after administration of 800 mg telithromycin. Telithromycin was analysed by a HPLC method.
Results: The mean telithromycin AUC for plasma was 247.8 mg h/L. For muscle tissue and subcutis a mean AUC of 34.97 and 52.35 mg h/L, respectively, could be found. The mean C_{max} of plasma, subcutis and muscle was 1.23, 0.18 and 0.16 mg/L, respectively.
Conclusion: A once daily dose of 800 mg telithromycin achieves concentrations in the interstitial space fluid which are sufficient to eradicate highly sensible pathogens like *S. pyogenes* but the dosage may lead to a therapeutic failure in treating infections caused by bacteria exceeding a MIC of 0.18 mg/L.

P1023 Pharmacokinetics of tigecycline in healthy adult volunteers and in subjects with renal impairment

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Background: TG is a novel glycylcycline antibiotic with an expanded spectrum of activity, including Gram-positive, Gram-negative, atypical and anaerobic pathogens. TG has also shown activity against pathogens resistant to other antibiotics. The goal of this analysis is to summarise the PK of TG in healthy volunteers and subjects with renal impairment.
Methods: PK data have been collected and analysed using non-compartmental methods for 144 subjects in four Phase 1 studies: three single IV dose (SD) and one multiple IV dose (MD) studies. This included six subjects with severe renal impairment (CrCl < 30 mL/min) and eight subjects with end stage renal disease (ESRD) receiving haemodialysis (HD). Doses ranged from 12.5 to 300 mg in SD and from 25 to 100 mg q12h in the MD study. TG was given under both fasting and fed conditions. Plasma and urine samples were collected and analysed using validated HPLC or LC/MS/MS methods. PK parameters, including maximum plasma concentration (C_{max}), half-life ($t_{1/2}$), area under the concentration-time curve (AUC), total clearance (CLt), renal clearance (CLr), and volume of distribution (*V*ss), were calculated.
Results: The plasma concentration-time profile was characterised by a steep decline in the distribution phase during the first 2 h, followed by a slower terminal phase. Steady state was reached in ~3 days. Healthy volunteer PK values listed in the table below are mean ± SD. With multiple doses, both C_{max} and AUC increased roughly in proportion with dose. CLr accounted for ~20% of CLt and less than 13% of TG was excreted unchanged in urine. CLt was reduced by ~20% in subjects with severe renal impairment or ESRD. TG was not removed by HD. Results of an SD age and gender study concluded C_{max} was lowest in young men and highest in elderly women (26% difference) and AUC was higher in young women than in young men (21% difference), with only a 4% difference between elderly women and men. At the target clinical dose of 100 mg load infused over 30–60 min followed by 50 mg q12h, C_{max} and AUCss (mean ± SD) were 621 ± 93 ng/mL and 3069 ± 381 ng h/mL, respectively.

	Dose (mg), with MD given q 12h						
Pk parameter	12.5	25	50	75	100	200	300
SD CLt (L/hr/kg)	0.29 ± 0.20 ($n = 6$)	0.20 ± 0.10 ($n = 6$)	0.28 ± 0.04 ($n = 6$)	0.29 ± 0.04 ($n = 6$)	0.30 ± 0.08 ($n = 57$)	0.23 ± 0.04 ($n = 24$)	0.25 ± 0.03 ($n = 12$)
MD CLt (L/hr/kg)	···	0.20 ± 0.04 ($n = 5$)	0.20 ± 0.02 ($n = 5$)	···	0.24 ± 0.045 ($n = 3$)	···	···
SD AUC® (ng h/mL)	753 ± 515 ($n = 6$)	2255 ± 1023 ($n = 6$)	2558 ± 534 ($n = 6$)	3658 ± 1003 ($n = 6$)	4872 ± 1405 ($n = 57$)	1321 ± 2796 ($n = 24$)	17294 ± 2176 ($n = 12$)
MD AUC® (ng h/mL)	···	1482 ± 259 ($n = 5$)	3069 ± 381 ($n = 5$)	···	4980 ± 925 ($n = 3$)	···	···
SD $t_{1/2}$(h)	11 ± 10 ($n = 6$)	32 ± 20 ($n = 6$)	18 ± 3.6 ($n = 6$)	22 ± 5.3 ($n = 6$)	22 ± 10 ($n = 57$)	52 ± 12 ($n = 24$)	44 ± 7.8 ($n = 12$)
MD $t_{1/2}$(h)	···	49 ± 35 ($n = 5$)	37 ± 12 ($n = 5$)	···	66 ± 23 ($n = 3$)	···	
SD Vss (L/kg)	2.8 ± 0.95 ($n = 6$)	6.4 ± 1.3 ($n = 6$)	6.5 ± 2.0 ($n = 6$)	7.5 ± 0.77 ($n = 6$)	6.8 ± 2.5 ($n = 57$)	13 ± 3.3 ($n = 24$)	12 ± 2.4 ($n = 12$)
MD Vss (L/kg)	···	4.0 ± 1.2 (n=5)	2.4 ± 0.66 (n=5)	···	2.9 ± 1.4 (n=3)	···	···
SD C_{max}® after 1 hr infusion (ng/mL)	109 ± 11 ($n = 6$)	252 ± 63 ($n = 6$)	383 ± 64 ($n = 6$)	566 ± 78 ($n = 6$)	927 ± 224 ($n = 57$)	1787 ± 525 ($n = 18$)	2817 ± 479 ($n = 6$)
MD C_{max}® after 1 hr infusion (ng/mL)	···	324 ± 54 ($n = 5$)	621 ± 93 ($n = 5$)	···	1173 ± 176 ($n = 3$)	···	···

Conclusions: TG exhibited approximate linear PK across all dose ranges evaluated in multiple-dose studies. TG has a long $t_{1/2}$ with a high *V*ss, indicating extensive tissue distribution. PK parameters were not significantly affected by food, age, or gender. TG is currently being developed for the treatment of complicated skin/soft tissue and intra-abdominal infections.

P1024 Comparative pharmacodynamics of ABT492 and ciprofloxacin (CIP) with *Escherichia coli* and *Pseudomonas aeruginosa* in an *in vitro* dynamic model

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Objective: To compare the pharmacodynamics of ABT492 relative to CIP, the kinetics of killing of Gram-negative organisms were studied using a dynamic model that simulates human pharmacokinetics of the fluoroquinolones.
Methods: Clinical isolates of *E. coli* and *P. aeruginosa* (MICABTs 0.01 and 0.12; MICCIPs 0.01 and 0.19 mg/L, respectively) were exposed to bi-exponentially decreasing concentrations of ABT492 (T1/2, alpha 2.1 h, T1/2, beta 23 h) and mono-exponentially decreasing concentrations of CIP (T1/2 4 h). Single dose simulations were performed with ABT492 vs. two 12-h doses CIP at different ratios of the area under the curve (AUC) to MIC, which varied from 60 to 480 h. Both *E. coli* and *P. aeruginosa* were also exposed to the clinically achievable AUC/MIC ratios of ABT492 (1740 and 140 h, respectively) and CIP (2200 and 120 h, respectively) that correspond to a 400-mg dose of ABT492 and two 500-mg doses of CIP. In addition, an 800-mg dose of ABT492 was simulated against *P. aeruginosa* as a single dose (AUC/MIC 280 h) and as two 400-mg doses given with a 12-h interval (AUC/MIC 2 × 140 h).
Results: The maximal reductions in the starting inoculum of antibiotic-exposed *E. coli* and *P. aeruginosa* were greater with ABT492 than with CIP at most of the simulated AUC/MICs, but the times

to regrowth were shorter with ABT492. Species-independent AUC/MIC relationships of the intensity of the antimicrobial effect (IE – area between the control growth and the time-kill/regrowth curves) were specific for ABT492 and CIP. Despite greater IEs produced by CIP at a given AUC/MIC ratio (from 120 to 480 h), the effect of the clinically achievable AUC/MIC of ABT492 (1740 h) on *E. coli* was more pronounced than the respective AUC/MIC of CIP (2200 h). With *P. aeruginosa*, a 140-h AUC/MIC of ABT492 (400 mg as a single dose) provided an 1.8-fold smaller IE than a 120-h AUC/MIC of CIP. However, two 12-h doses of ABT492 (AUC/MIC 2×140 h) were even more efficient than CIP.

Conclusion: These findings predict comparable efficacies of clinically achievable AUC/MICs of ABT492 and CIP against *E. coli* (q.d. vs. b.i.d. quinolone dosing) and *P. aeruginosa* (b.i.d. dosing) but suggest lower efficacy of q.d. ABT492 against *P. aeruginosa*.

P1025 ABT492 vs. levofloxacin in an *in vitro* dynamic model: comparative pharmacodynamics with differentially susceptible *Staphylococcus aureus* and the relative ability to prevent the selection of resistant mutants

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Objective: To compare the kinetics of killing/regrowth of differentially susceptible clinical isolates of *Staphylococcus aureus* exposed to ABT492 and levofloxacin and to explore their relative abilities to prevent the selection of resistant mutants.

Methods: Three clinical isolates of *S. aureus* including two ciprofloxacin-susceptible *S. aureus* 201 and 480 and a ciprofloxacin-resistant *S. aureus* 866 were exposed to clinically achievable ratios of area under the curve (AUC) to MIC in a dynamic model that simulated human pharmacokinetics of ABT492 (400 mg) and levofloxacin (500 mg) as a single dose. In addition, *S. aureus* 201 was exposed to single and multiple doses of ABT492 and levofloxacin (both q.d. for 3 days) over wide ranges of the 24-h AUC/MIC (AUC24/MIC) including clinically achievable AUC24/MIC ratios.

Results: With each isolate, ABT492 at the clinically achievable AUC/MICs produced greater anti-staphylococcal effects than levofloxacin. Areas between the control growth and the time-kill curves (ABBC in single dose simulations and the sum of ABBCs determined after the first, second and third dosing in multiple dose simulations – ABBC1 + 2 + 3) were larger with ABT492 than levofloxacin. Moreover, at comparable AUC/MICs and AUC24/MICs maximal reductions in the starting inoculum of ABT492-exposed *S. aureus* were more pronounced than with levofloxacin. Loss in the susceptibility of *S. aureus* 201 exposed to both ABT492 and levofloxacin depended on the simulated AUC24/MIC. Although the maximal increase in MIC (MICfinal) related to its initial value (MICinitial) was seen at the higher AUC24/MIC ratio of ABT492 (120 h) than levofloxacin (50 h), similar AUC24/MICs (240 and 200 h, respectively) were protective against the selection of resistant *S. aureus*. These threshold values are readily achievable with 400 mg ABT492 (AUC24/MIC 870 h) but not with 500 mg levofloxacin (AUC24/MIC 70 h).

Conclusion: Overall, these findings predict greater efficacy of clinically achievable AUC/MIC (or AUC24/MIC) of ABT492 both in terms of the anti-staphylococcal effect and prevention of the selection of resistant mutants.

P1026 Pharmacodynamic activity of iclaprim vs. resistant *Streptococcus pneumoniae* using an *in vitro* model

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Objectives: Iclaprim (formerly AR-100) is a novel investigational diaminopyrimidine currently completing Phase II clinical trials for the treatment of 'difficult-to-treat' nosocomial infections. The purpose of this study was to assess the pharmacodynamic activity of Iclaprim using an *in vitro* pharmacodynamic model (IVPM) against *S. pneumoniae* collected from across Canada.

Methods: Six *S. pneumoniae* isolates with varying susceptibilities to penicillin, erythromycin, levofloxacin, trimethoprim/sulfamethoxazole and Iclaprim. The IVPM was inoculated with 1×106 CFU/ml and Iclaprim was dosed at 0 and 12 h to simulate a C_{pmax} of 3.5 μg/mL and $t_{1/2}$ of 3 h. Sampling was performed over 24 h to assess viable growth.

Results: Of the six *S. pneumoniae* studied, three demonstrated Iclaprim MIC of 0.03 μg/mL and three were 1 μg/mL. Resistant phenotypes included penicillin (MIC 2 μg/mL), erythromycin (MIC 1 μg/mL), levofloxacin (MIC 8 μg/mL) and trimethoprim/sulfamethoxazole (MIC 4 μg/mL). At 6, 12 and 24 h, Iclaprim demonstrated the following bacterial reduction (range of log 10 killing) 1–2.8, 1.3–2.6 and 1.8–2.9, respectively. No difference in bacterial reduction was noted irrespective of antibiotic resistant phenotypes including the trimethoprim–sulphamethoxaxole resistant strains. No bacterial re-growth was observed during the study period.

Conclusion: Iclaprim demonstrated activity against *S. pneumoniae* including penicillin, erythromycin, levofloxacin and trimethoprim/sulphamethoxazole resistant strains. Iclaprim demonstrates promising activity against *S. pneumoniae* resistant to other antibiotic classes.

P1027 Single dose pharmacokinetics (PK) of telavancin (TLV) in healthy elderly subjects

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Background: TLV is a novel antibiotic that exerts rapid concentration dependent bactericidal activity against clinically important gram-positive bacteria, including methicillin-resistant *S. aureus* and penicillin-resistant pneumococci.

Objective: To determine single dose PK of TLV in healthy male and female elderly subjects and compare the findings to those from a group of young healthy male and female volunteers from another study.

Methods: 16 healthy elderly subjects (average age 71 years, range 66–83) received TLV (10 mg/kg) intravenously over 60 min. Tolerability was assessed by collecting adverse events. Blood samples were obtained at specified intervals and analysed for TLV concentration with a validated LC/MS/MS assay. PK parameters were determined using non-compartmental methods.

Results: Mean (SD) PK parameters for TLV in male and female elderly subjects are shown below. The PKs of TLV were not different between genders. Compared with young subjects given 7.5 mg/kg TLV over 1 h, the average CL value of TLV from plasma was similar in young and elderly subjects (12 mL/h/kg), whereas *V*dss was higher in elderly than young subjects (167 vs. 100 mL/kg) and $t_{1/2}$ was longer in the elderly (11 vs. 7 h). Adverse events included altered taste ($n = 2$), flushing with postural hypotension ($n = 1$), headache ($n = 2$), dizziness ($n = 1$) and paresthesia of the throat ($n = 1$). All of these events were transient and of mild intensity. Subjects with adverse events did not have different PK parameters than those who did not report them.

Gender	C_{max} (μg/mL)	AUC (μg*hr/mL)	CL (mL/hr/kg)	*V*dss (mL/kg)	$T_{1/2}$ (hr)
Male ($n = 8$)	84.7 (8.3)	820 (94)	12 (2)	173 (12)	11.0 (1.7)
Female ($n = 8$)	90.8 (10.7)	858 (92)	12 (1)	161 (13)	10.6 (1.1)

Conclusions: Age does not affect the CL of TLV, but was associated with more extensive distribution and longer elimination $t_{1/2}$. In addition, gender did not influence the PK of TLV. No dose adjustments of TLV are needed in elderly patients with normal renal function.

P1028 Single dose pharmacokinetics (PK) of telavancin (TLV) in subjects with renal dysfunction

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Background: TLV is a novel antibiotic with multiple mechanisms of action that exerts rapid and concentration-dependent bactericidal activity against clinically important Gram-positive bacteria, including methicillin-resistant *S. aureus* and penicillin-resistant pneumococci. Previous studies in man have determined that the primary route of elimination of TLV is the kidney.
Objective: To determine the single dose PK of TLV in subjects with renal dysfunction (creatinine clearance CLcr <80 mL/min) and compare the findings to a group of healthy volunteers from another study (7.5 mg/kg over 1 h).
Methods: 10 (eight males, two females) subjects with renal impairment received a single 7.5-mg/kg dose of TLV intravenously over 60 min. Five subjects had mild impairment (CLcr 51–80 mL/min), three had moderate impairment (30–50 mL/min) and two had severe renal impairment (<30 mL/min). Blood samples were obtained at specified intervals and assayed for TLV with a validated LC/MS/MS assay. PK parameters were determined using non-compartmental methods.
Results: The average preliminary values for PK parameters for TLV in subjects with renal dysfunction are shown below. Mean PK values for TLV in healthy subjects were: C_{max} (89 μg/mL), AUC (606 μg h/mL), CL (11 mL/h/kg), *V*dss (101 mL/kg), and $t_{1/2}$ (7.2 h). Severe renal dysfunction was associated with slightly higher AUC values, longer CL and $t_{1/2}$ values compared with subjects with mild to moderate renal impairment and healthy volunteers. In subjects with renal impairment there was a significant correlation ($r = 0.88$) between CL of TLV and CLcr. One subject (mild) developed 'red man syndrome' and received only two-thirds of the intended dose and was excluded from the PK analysis. The other subjects tolerated TLV well.

Degree of dysfunction	CLcr (mL/min)	C_{max} (μg/mL)	AUC (μg/h/mL)	CL (mL/h/kg)	*V*dcc (mL/kg)	$t_{1/2}$ (h)
Mild ($n = 4$)	71	68.1	537	11	165	11.8
Moderate ($n = 3$)	39	74.1	628	9	149	13.2
Severe ($n = 2$)	24	73.5	773	6	148	17.9

Conclusions: Dose reductions of TLV may be needed in patients with moderate to severe renal impairment who have serious infections.

P1029 Efficacy of telavancin (TD-6424), a rapidly bactericidal agent with multiple mechanisms of action, in a murine model of MRSA pneumonia

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Objectives: Telavancin (TD-6424) is a novel lipidated glycopeptide currently in Phase 2 trials, which possesses rapid and concentration-dependent bactericidal activity against clinically relevant gram-positive organisms including methicillin-resistant *Staphylococcus aureus* (MRSA). In the present study, the efficacy of telavancin (TLV) was compared with vancomycin (VAN) and linezolid (LIN), against MRSA 33591 in a murine pneumonia model.
Methods: Immunocompromised mice (Balb/c, eight per group) were inoculated intranasally with ~log 7 of MRSA 33591. At 24-h after inoculation, mice were dosed intravenously with either no drug (control) or two doses (q 12-h) of TLV (40 mg/kg), VAN (80 mg/kg) or LIN (110 mg/kg). The doses of the three test drugs were chosen to equate to human exposure (24-h AUC) at their respective clinical doses. At 24-h after treatment initiation, lungs were harvested and processed to quantify bacterial titres (log CFU/g).
Results: At 1-h post-inoculation, the lung bacterial titre was 5.9 ± 0.2 log CFU/g. The pre-treatment titre (24-h post-inoculation) was 7.2 ± 0.6 log CFU/g. The table below shows the lung bacterial titre and % survival at 24-h after either no treatment (control) or treatment with TLV, VAN or LIN.

	Control	TLV	VAN	LIN
Lung titre (log_{10}CFU/g ± SD)	8.4 ± 0.2	4.4 ± 0.7 *#	7.0 ± 1.8	7.6 ± 1.6
% Survival	38%	100%	88%	88%

*P<0.05 vs Control
#P<0.05 vs VAN and LIN

Conclusions: Telavancin is efficacious and produces significantly greater reduction in lung titre than vancomycin and linezolid in the MRSA murine pneumonia model. Telavancin appears to be a promising new antibacterial for the treatment of MRSA-induced pneumonia.

P1030 Influence of gender on the pharmacokinetics of BAL9141 after intravenous infusion of Pro-drug BAL5788

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Objectives: BAL5788 is the water-soluble pro-drug of BAL9141, a novel cephalosporin with broad-spectrum bactericidal activity against Gram-positive and Gram-negative pathogens, including MRSA, VRSA, and penicillin-resistant *Streptococcus pneumoniae*. The present study was specifically designed to characterise the pharmacokinetic properties of the active drug BAL9141 in male and female subjects.
Methods: The pharmacokinetics of active drug BAL9141 were investigated in 12 male and 12 female subjects. Each subject received a single 30-min constant-rate intravenous infusion of BAL5788 (equivalent to 750 mg of BAL9141). Blood and urine samples were collected at appropriate intervals up to 24 h after start of infusion. Pharmacokinetic parameters were derived from plasma concentrations of BAL9141 by non-compartmental analysis.
Results: At the end of the 30-min infusion, plasma C_{max}-values of BAL9141 reached 65.6 μg/mL in males and 79.3 μg/mL in females. The corresponding AUCinf-values were 137 and 157 μg h/mL, respectively. Elimination half-lives ($t_{1/2}$ (b)) of BAL9141 amounted to 3.4 h in males and 2.8 h in females. Total systemic clearance (CLS) was 5.5 L/h in males and 4.9 L/h in females. The largest gender difference was in the volume of distribution (VSS), which amounted to 17 L in males and 12 L in females. All of these gender differences were statistically significant ($P < 0.05$). However, if VSS and CLS were corrected for body weight, and if AUC and C_{max} were normalised to doses of '1 mg/kg body weight', no significant gender differences were found for VSS ($P = 0.13$) or C_{max} ($P = 0.27$). Urinary recovery of BAL9141 amounted to 92% of the dose in males and to 96% in females.
Conclusion: Systemic exposure to BAL9141 was approximately 15% higher in female subjects compared with male subjects and was primarily related to the lower body weight/size and hence

smaller volume of distribution (VSS) in females. These small differences are clinically not relevant, and therefore, no dose adjustment is required in females.

P1031 BAL5788 in patients with complicated skin and skin structure infections caused by Gram-positive pathogens including methicillin-resistant *Staphylococcus* species. Interim pharmacokinetic results from 20 patients

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Objectives: BAL5788 (pro-drug) is a new cephalosporin with broad-spectrum bactericidal activity against Gram-positive and Gram-negative pathogens, including methicillin-resistant *Staphylococci* and penicillin-resistant *Streptococcus pneumonia*. Studies in animals demonstrated that time above MIC ($T > MIC$) for BAL9141 (active drug) is predictive for antibacterial efficacy. Based on multiple dose pharmacokinetic studies in healthy volunteers (HV), as well as Monte Carlo simulations, a dose of 750 mg bid resulted in a $T > MIC$ largely greater than 60% for a MIC90 of 2 μg/mL in MRSS. BAL5788 is currently under evaluation in Complicated Skin and Skin Structure Infections (cSSSI) at a dose of 750 mg bid. Interim pharmacokinetic data from 20 patients (P) are available with the objective to confirm the dose selected.
Method: Patients were treated with 750 mg equivalent BAL9141 (given as BAL5788 as a 30-min infusion) bid for at least 7 days. Serial blood samples were collected during the second administration (Study days 1 or 2) and on Study day 7 during the first daily administration. Plasma concentrations of BAL9141 were determined using a specific LC/MS assay.
Results: The plasma concentration-time profiles of BAL9141 were similar on Study Days 2 and 7 indicating in P the lack of accumulation after repeated administrations. At steady state (SS) on Study Day 7, mean ($\pm$SD) C_{max}-values (close to the end of the infusion) of 64.4 μg/mL ($\pm$31.9, $n = 20$) were obtained, similar to the reported mean C_{max} of 60.6 μg/mL ($\pm$9.99, $n = 6$) in HV at SS. The mean ($\pm$SD) AUClast in P was 136 μg h/mL ($\pm$34.0), also comparable to HV. At 10 h after start of the infusion (i.e. 80% of a 12-h dose interval) the mean ($\pm$SD) plasma concentrations of BAL9141 were 2.31 μg/mL ($\pm$1.31) .The inter- and intra-subject variability in P was low (<30%) and similar to HV.
Conclusion: The interim pharmacokinetics of BAL9141 confirmed that bid doses of 750 mg in patients resulted in plasma concentrations above a MIC90 of 2 μg/mL for >80% of the dosing interval and are therefore predicted to be efficacious in cSSSI with MRSS.

P1032 Dose adjustment in subjects with normal and impaired renal function based on the pharmacokinetics of BAL5788

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Objectives: BAL5788 is the pro-drug of BAL9141, a novel cephalosporin with broad-spectrum bactericidal activity including methicillin-resistant *Staphylococci* and penicillin-resistant *Streptococcus pneumonia*. After intravenous infusion, cleavage of the prodrug to BAL9141 occurred rapidly and quantitatively. BAL9141 was predominantly excreted in urine. Therefore the present study was designed to assess the pharmacokinetics of BAL9141, to suggest dose-adjustment and to evaluate methods to optimally classify the subjects.
Methods: Pharmacokinetics of BAL9141 were investigated in 20 healthy male subjects ($n = 5$/group) with normal renal function and mild, moderate, or severe renal impairment (respectively, creatinine clearance CLCR >80, 51–80, 31–50, and <30 mL/min). Each subject received a single intravenous dose of BAL5788 (equivalent to 250 mg BAL9141) infused over 30 min. CLCR was measured by 24-h urinary excretion and was calculated using the Cockroft & Gault and Levey equations.
Results: Renal function affected clearance, elimination half-life, and urinary excretion of BAL9141. Based on the linear correlation between measured creatinine clearance and systemic drug clearance and taking as target systemic exposure a MIC value of 4 μg/mL BAL9141 exceeded for at least 6 h (corresponding to 50% of a 12-h dose interval) the following dose adjustments were suggested: subjects with a CLCR of >80 mL/min: 750 mg twice daily; subjects with a CLCR of 30–80 mL/min: 500 mg twice daily; and subjects with a CLCR of <30 mL/min: 250 mg once daily. The classification of renal function was reliable when done using the Cockroft equation down to CLCR of 30 mL/min. For more severe renal impairment the Levey equation was more accurate.
Conclusions: In subjects with normal and impaired renal function, systemic clearance of BAL9141 correlated well with creatinine clearance. Therefore, dose adjustments for treatment of target pathogens can be proposed based on creatinine clearance. Classification of renal function and appropriate dose adjustments can be reliably based on the equation of Cockroft & Gault for CLCR >30 mL/min using serum creatinine concentrations, body weight and age.

P1033 Pharmacokinetics of BAL4815, a new azole antifungal, after administration of single ascending intravenous doses of its pro-drug BAL8557

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Objective: BAL8557 is a water-soluble pro-drug, rapidly converted in plasma to the active azole BAL4815. *In vitro*, BAL4815 showed broad-spectrum antifungal activity against all major opportunistic fungi and the true pathogenic fungi, including fluconazole-resistant strains. The pro-drug BAL8557 is specifically suited for intravenous and oral administration. The objective of the present study was the first assessment of the intravenous pharmacokinetics of BAL8557/BAL4815 in human, after single ascending doses.
Methods: In this double-blind, placebo-controlled study, 18 male subjects were randomly assigned to receive single ascending 1h-constant rate intravenous infusions of 50, 100, or 200 mg of BAL4815 equivalents in the form of BAL8557. Blood samples were collected up to 960 h after infusion and pharmacokinetic parameters were estimated from plasma concentrations of BAL4815 by non-compartmental analysis.
Results: At the end of the 1-h infusion of 50, 100, and 200 mg, C_{max}-values of BAL4815 reached 0.446, 1.03, and 2.47 μg/mL. The corresponding AUCinf-values were 11.3, 26.6, and 73.2 μg h/mL. Concentrations of BAL4815 seemed to increase slightly more than dose-proportionally. Mean elimination half-lives reached 77, 105, and 79 h and appeared to be independent of the dose. The volume of distribution (VSS) amounted to 305–496 L and systemic clearance was low and reached only 2.8–5.0 L/h. Urinary recovery of BAL4815 was low (<0.4% of the dose).
Conclusion: BAL8557 was rapidly converted in plasma to BAL4815. High systemic concentrations of BAL4815 were reached for prolonged times. Non-renal excretion seems to be the predominant elimination pathway of BAL4815. Comparison of the systemic exposure to BAL4815 after intravenous administration with that after oral administration points to an excellent absolute bioavailability of the oral formulation.

P1034 Anidulafungin (ANID) pharmacokinetics are not affected by concomitant voriconazole (VORI)

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Objectives: Currently there is a great deal of interest in the combination of triazole and echinocandin antifungals for invasive

aspergillosis. However, little information exists on the pharmacokinetics when these agents are coadministered. ANID (Vicuron Pharmaceuticals) is a novel semi-synthetic echinocandin in late-stage development for the treatment of serious fungal infections. VORI (Pfizer; VFEND®) is an extended-spectrum triazole antifungal agent with demonstrated efficacy against invasive aspergillosis and candidiasis. A study was done to assess a possible pharmacokinetic interaction following co-administration of VORI and ANID.

Methods: A blinded, randomised, multiple-dose, cross-over, two-way pharmacokinetic interaction study was conducted in healthy subjects. Subjects received treatments in a random sequence: IV ANID with an oral placebo, IV placebo with oral VORI, and IV ANID with oral VORI. Treatments were separated by a greater than 10-day washout. ANID was given as 200 mg IV on Day 1, then 100 mg IV/day (Days 2–4). VORI was given PO as 400 mg q 12 h on Day 1, then 200 mg PO q 12 h (Days 2–4). Pharmacokinetic parameters for ANID and VORI were determined at steady-state from samples collected after the Day 4 dose of each treatment. Maximum plasma concentration (C_{max}) and area under the plasma concentration vs. time curve (AUC) were determined for ANID and VORI administered alone and in combination.

Results: Eighteen healthy males were enrolled in the study. Seventeen of these subjects, 20–40 years of age, completed all treatments. Steady-state ANID and VORI pharmacokinetic parameters were calculated and summarised in this analysis; ANID pharmacokinetic parameters are shown below (mean ± SD). No dose-limiting toxicities or serious adverse events occurred in the study.

Treatment	C_{max} (mg/L)	AUC (mg.h/L)
Anidulafungin	7.87 (1.64)	120 (24)
Anidulafungin+Voriconazole	7.89 (1.35)	117 (22)

Conclusions: The pharmacokinetic parameters are not affected when ANID and VORI are administered concomitantly.

P1035 Population pharmacokinetics confirms absence of anidulafungin drug–drug interactions

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Objectives: Anidulafungin (ANID) is an echinocandin in development for the treatment of serious fungal infections. It is chemically degraded, not metabolised, and not excreted renally; therefore ANID has low potential to interfere with the elimination of other drugs. A population pharmacokinetics (PK) analysis was performed to screen for potential drug interactions that may affect ANID PK.

Methods: Population PK analysis included 600 steady state drug concentrations from 225 patients with esophageal or invasive candidiasis, or aspergillosis. Patients received 50, 75, or 100 mg ANID/day. Mixed-effects models were evaluated in the NONMEM program. Concomitant medications were categorised metabolically as substrates, inducers, or inhibitors of cytochrome P450 and evaluated in the model. Rifampin, a known potent inducer, was included as a separate variable. Only those categories present in at least 10% of the sampled population were evaluated during the modelling process.

Results: A two-compartment model with first order elimination best fit the data. Interpatient variability (%CV) in clearance (CL) was estimated at 28%. Weight, gender, and study differences together accounted for variability <20% and were not clinically relevant in that dosage adjustments were not warranted. The steady state parameters for a typical 60-kg male patient with oesophageal candidiasis taking 50 mg/day were $C_{max} = 3.5$ mg/L, AUCss = 53 mg h/L, $Vss = 33.4$ L, $t_{1/2} = 25.6$ h, and CL = 0.934 L/h. No other covariates had any statistically significant effects on the model. Patients receiving no concomitant interacting medications, known inhibitors, and known inducers had CL (mean ± SD) values of 0.93 (0.29), 1.03 (0.34), and 0.95 (0.25) L/h, respectively. Co-administration of rifampin also showed no change in the PK (CL = 0.94 (0.27) L/h). Based on the mechanism of ANID elimination, mainly via chemical degradation, the lack of an effect due to concomitant medication usage was expected. For most of the concomitant medication categories (rifampin, inducer, inhibitor), there were adequate numbers of patients (>10% per category) to have detected any consistent, clinically meaningful changes.

Conclusions: ANID PK parameters in patients with serious fungal infections were predictable with low intersubject variability. No dosage adjustments of ANID are required for concomitant use with metabolic substrates, inducers, and inhibitors.

P1036 Assessment of the pharmacokinetics of anidulafungin in patients with invasive aspergillosis receiving concomitant liposomal amphotericin

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Objective: There is a great deal of interest in combination antifungal therapy for the treatment of aspergillosis. There are, however, little clinical PK data on the concomitant use of echinocandins and amphotericin. ANID is an echinocandin being studied for serious fungal infections. It is chemically degraded, not metabolised, and not excreted renally, therefore ANID has low potential to interfere with the elimination of other drugs. An investigation of potential clinical PK interactions was performed by comparing the PK of ANID in patients with IA receiving concomitant LAmB to other patient populations.

Methods: Patients with IA were enrolled in an open label non-comparative study of ANID and received a combination of intravenous ANID (100 mg/day) and LAmB (AmBisome(R), Gilead; 3–5 mg/kg/day). Patients were treated until the resolution of signs and symptoms for a maximum of 90 days. Plasma samples obtained at steady-state on separate days, at various sampling intervals post-dose, were assayed for ANID. Data were combined with data from patients with invasive and oesophageal candidiasis (CA), analysed using a population PK analysis (nonlinear mixed effects modelling), and PK parameters were compared.

Results: A two-compartment population PK model was established that described the steady-state PK of ANID. The model was developed using 600 ANID plasma concentrations collected from 225 patients from four recent patient studies. Seven patients with IA, receiving concomitant LAmB, were included in the analysis. Overall, the population PK model showed little impact due to any intrinsic or extrinsic factors, such as patient demography, concomitant medications, or disease. Patient weight and gender were found to be covariates on the model, but had little clinical importance, accounting for less than 20% of the intersubject variability. Patients with IA receiving concomitant LAmB had similar PK parameters as patients with CA who did not receive concomitant LAmB, as shown in the table (parameter means ± SD).

	CL (L/h)	Vss (L)	$t_{1/2}$ (h)
Patients w/IA	0.84 (0.23)	34.4 (1.7)	30.6 (7.2)
Patients w/CA	1.01 (0.35)	33.4 (4.8)	25.5 (7.4)

Conclusions: Patients with IA receiving concomitant LAmB had no observable differences in ANID PK compared with other patients. This is consistent with other screened factors, and consistent with the elimination mechanism of the drug.

P1037 Rifampicin and ritonavir do not affect the pharmacokinetics of micafungin (FK463), an echinocandin antifungal

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Objectives: Pharmacokinetic (PK) interactions between caspofungin, a recently marketed echinocandin, and rifampicin were reported. Two PK studies were performed to determine the effect of rifampicin or ritonavir on the single IV dose pharmacokinetics of micafungin.
Methods: The potential for drug interactions between micafungin and rifampicin or ritonavir was investigated in two phase I open-label studies in healthy male subjects. All subjects (rifampicin: $n = 24$; ritonavir: $n = 24$) received a single IV dose of 200 mg micafungin on Day 1, followed by a washout period of 4 days (ritonavir: 5 days). Subjects received oral doses of 600 mg rifampicin once-daily on Days 5–15 or 300 mg ritonavir twice-daily on Days 6–17. A second IV dose of 200 mg micafungin was co-administered on Day 12 (ritonavir: Day 10).
Results: The geometric mean ratio (90% CI) for micafungin plasma AUC(0–inf) on the day of co-administration relative to administration alone was 1.02 (0.977, 1.06) for rifampicin and 1.02 (0.993, 1.05) for ritonavir. The corresponding ratio of C_{max} were 0.974 (0.946, 1.00) and 1.04 (0.999, 1.07), respectively.
Conclusions: Multiple oral doses of rifampicin, a potent inducer of CYP3A4, or ritonavir, a potent inhibitor of CYP3A4, had no effect on the pharmacokinetics of micafungin. Co-administration of rifampicin or ritonavir with micafungin did not change the systemic exposure of micafungin compared with single IV doses given alone.

P1038 Pharmacokinetics of itraconazole and OH-itraconazole as a nanocrystal formulation in healthy volunteers after single and multiple doses

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Background: Until recently, itraconazole was available as an oral agent only. Because of the water insolubility of itraconazole, formulations for intravenous use have met with great difficulties. So far, the only formulation used for intravenous administration is a 40% hydroxypropyl-beta-cyclodextrin solution (HBC). An alternative method to administer itraconazole (IT) could be the use of a nanocrystal formulation (NCF). However, studies using this approach in several animal species revealed that the nanocrystals are specifically trapped in Kupffer cells in the liver and the spleen. This could result in significant changes in the pharmacokinetics of IT.
Methods: Two pharmacokinetic studies in volunteers were performed. Study 1 was a single ascending dose study (SAD) with doses planned to range from 50 to 500 mg ($n = 9$); Study 2 was a single and multiple dose ascending study (MAD 100, 200 and 300 mg, $n = 4$) of IT as the NCF and one dose level as HBC (200 mg, $n = 4$). Samples were collected immediately before starting the infusion, at 0.5 and 1 and 2 h during infusion when applicable, at 0, 0.08, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8 h after the end of infusion and at 24, 32, 72, 96 and 168 h after the start of infusion and analysed by HPLC.
Results: The results of both the SAD as well as the MAD study indicated that there was a dose dependency in half-life of the NCF. The half-life of IT increased from 44 h (100 mg dose) to more than 150 h (300 mg) once steady state was achieved. Similar dose dependent effects were observed for the metabolite; the AUC was also dose dependent. Comparing the 200 mg treatments of the two formulations, mean peak plasma concentrations during infusion of NFI were substantially higher compared with itraconazole HCI. However, during the first 30 min after infusion of NFI there was a more rapid concentration and, as a consequence, the difference between the two 200 mg formulations for other pharmacokinetic parameters was less pronounced or not significant at all. Both formulations were comparable with respect to the terminal half-life, both after single dose as well as during steady state.
Conclusion: There is significant a dose dependent increase in half life for the nanocrystal formulation. This might be due to trapping of the crystals in liver and spleen cells acting as a deep compartment. High dose nanocrystal formulation may especially advantageous for long term therapy and profylaxis.

P1039 Pharmacokinetics and tissue penetration of linezolid in patients with diabetic foot infection

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Objectives: DFI continues to be a major problem in diabetic patients. Linezolid (L) has microbiological activity against the predominant pathogens causing DFI. For clinical efficacy sufficient penetration of antimicrobial agents into perinecrotic tissue is necessary. Data on plasma pharmacokinetic of L in diabetic patients are not available.
Methods: 14 patients with a mean age of 65 years (range 53–77) with DFI (Wagner grade III) were included into the study and received at least five oral doses 600 mg L, given every 12 h. At steady state blood and tissue-samples from the perinecrotic area were taken. Tissue penetration and plasma pharmacokinetic parameters were determined. Concentrations of L were measured by high-pressure liquid chromatography (HPLC).
Results: Mean pharmacokinetic parameters of L were: plasma peak concentration (C_{max}) 13.7 ± 3.8 μg/mL attained at a time of 2.8 ± 1.8 h (T_{max}); AUC 0–12 112.3 ± 32 μg h/mL; plasma elimination half-life ($t_{1/2}$) 7.8 ± 4 h. Based on MIC of 4 mg/L calculated PK/PD-values were: AUC24/MIC ratio 56 ± 16; time above MIC 100% in 11 patients and in remaining three patients 67–83%. The mean concentration of L in the inflamed perinecrotic tissue after 3 h was 10.6 ± 4 μg/mL (11 patients); after 6 h, 6.8 μg/mL (three patients); after 7 h 5.0 μg/mL (one patient), this corresponds to 90, 70 and 93% of plasma levels.
Conclusion: L exhibits good penetration into inflamed perinecrotic tissue, with concentrations 3–7 h after dosing exceeding the MIC of susceptible pathogens. Compared with pharmacokinetic data of L in healthy volunteers with similar dosage, C_{max} and AUC tend to be decreased whereas T_{max} and $t_{1/2}$ tend to be increased in diabetic patients. The data presented confirm that L could be successfully used in the treatment of DFI.

P1040 Evaluation of tigecycline, linezolid, quinupristin-dalfopristin, arbekacin and daptomycin alone and in combination with various antimicrobials against two clinical strains of vancomycin-resistant *Staphylococcus aureus* in an *in vitro* pharmacodynamic model

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Objectives: Increase in vancomycin usage has led to the development of resistance to this drug due to selective pressure. This was first observed with the emergence of Glycopeptide-Intermediate *Staphylococcus aureus* (GISA) in 1996 and with Vancomycin-Resistant *Staphylococcus aureus* (VRSA) reported in 2002. Treatment options for glycopeptide resistant strains limited and efforts to investigate various combination therapies against VRSA are warranted.
Methods: Two clinical strains of VRSA-MI (Michigan) and VRSA-PA (Pennsylvania) and one presumptive parent-MRSA from Michigan (pMRSA) were evaluated, and were obtained from the Detroit Medical Center, Detroit, MI, and the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA) program. MIC and MBC were performed by NCCLS. An *in vitro* Pharmacodynamic Model (IVPM) with a starting inoculum of 106 CFU/mL was used for all experiments. Human pharmacokinetic regimen simulations were: tigecycline (T) 100 mg q24h, linezolid (L) 600 mg q12h, arbekacin (A) 100 mg q12h, daptomycin (D)

6 mg/kg q24h, and vancomycin (V) 1 g q12h. Quinupristin–dalfopristin (QD) was simulated as separate components at 7.5 mg/kg q8h. Bacterial density was measured over 48 h.
Results: MICs for T, L, QD, A, D, and V for both VRSA-MI/pMRSA are 0.5/0.5, 1/1, 0.5/0.25, 0.5/0.5, 0.25/0.25, and 1024/0.5 mg/L, respectively. MICs for VRSA-PA strain are 2, 0.25, and 32 mg/L. T alone demonstrated bacteriostatic activity against VRSA. L, QD, A, and D alone exhibited bactericidal activity (99.9% kill) by 48 h against VRSA. However, no combination exhibited significant activity vs. VRSA.
Conclusions: L, QD, A, and D were highly active against VRSA. Further investigations with combination therapies are warranted.

P1041 Evaluation of cefepime, arbekacin, daptomycin, gentamicin, tobramycin, and tigecycline alone and in combination against methicillin-resistant *Staphylococcus aureus* in an *in vitro* pharmacodynamic infection model

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Objectives: Over the last two decades, antimicrobial resistance among Gram-positive bacteria has become widespread in the intensive care unit. Treatment options are limited; therefore, efforts to develop new treatment regimens in the critical care population are warranted. We investigated various regimens of cefepime alone and in combination therapies against two clinical Methicillin-Resistant *Staphylococcus aureus* (MRSA) isolates (R2481 and R2484) in an *in vitro* pharmacodynamic model *in vitro* Pharmacodynamic Infection Model (IVPM).
Methods: Two clinical MRSA isolates (Beaumont Hospital, Royal Oak, MI) were evaluated. An IVPM was used for all experiments. Human pharmacokinetic regimen simulations were: cefepime (C) 2 g q8 and 12 h, cefepime continuous infusion (CCI) 2 g loading dose followed by 4 g alone or in combination with gentamicin (G) 1.5 mg/kg q12h (3 and 6 mg/L), arbekacin (A) 100 mg q12h, tobramycin (TB) 3 mg/kg q12h (3 and 6 mg/L), linezolid (L) 600 mg q12h, tigecycline (T) 100 mg q24h, and daptomycin (D) 6 mg/kg q24h. Bacterial density was measured over 48 h. MIC and MBC were performed by NCCLS.
Results: MICs for C, G, A, TB, L, T, and D for the two clinical MRSA isolates (R2481 and R2484) were 4/4, 0.25/0.5, 0.5/0.125, 128/0.5, 2/4, 0.25/0.25, and 0.0625/0.125 μg/mL, respectively. There was a trend for more activity with CCI vs. q8h vs. q12h. At 48 h, combinations of C q12h + A demonstrated enhanced kill (-2.90 log 0 decrease) against R2484 ($P < 0.05$). C q12h + G3 mg/L demonstrated enhanced kill (-3.21 and -4.32 log 10 decrease) against R2481 and R2484, respectively ($P < 0.05$). C q8h + G3 mg/L demonstrated enhanced kill (-3.06 and -2.05 log 10 decrease) against R2481 and R2484, respectively. T alone demonstrated bacteriostatic activity against these isolates. However, T + CCI demonstrated improved kill against R2481 but no difference with R2484.
Conclusions: Overall, the most potent combinations noted were C q8 and 12 h + G3 mg/L and C q12h + A against both clinical isolates. An improved kill were noted with these combinations: CCI + G3, CCI + A, and CCI + T. D alone was highly active against both clinical MRSA isolates. Further investigations with combination therapies are warranted.

P1042 Simultaneous methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecalis* infections in an *in vitro* pharmacodynamic model: a comparison of daptomycin, arbekacin, linezolid and tigecycline activities

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Objective: Mixed pathogen infections represent a serious therapeutic challenge and choosing appropriate therapy, especially if the pathogens are multi-drug resistant (MDR) are difficult. We inoculated an *In vitro* pharmacodynamic model (IVPD) simultaneously with two pathogens to determine activity.
Methods: daptomycin (D), arbekacin (A), linezolid (L), tigecycline (T), and vancomycin (V) MICs were determined according to NCCLS guidelines. A clinical isolate of methicillin resistant *Staphylococcus aureus* (MRSA-494) and vancomycin resistant *Enterococcus faecalis* (VREFc-R2526) was used in an IVPD model at 7 log 10 CFU/g to mimic a mixed infection (MI). Simulated regimens were D 6 mg/kg/day, A 100 mg q12h, T 100 mg q24, and L 600 mg q12h . Each sample was plated on an ampicillin (Am), and vancomycin (V) TSA plate at 8× the MIC to isolate the respective isolate. Simulations were performed in duplicate, bacterial quantification occurred over 48 h and potential for resistance was evaluated.
Results: MICs for MRSA vs. D, Am, A, L, T, and V were 0.25, 512, 0.25, 2, 0.125, and 0.25 mg/L; for VREFc vs. D, Am, A, L, T, and V were 1, 1, 64, 2, 0.125, and >256, respectively. In the MI model, stunting of MRSAs growth was noted (1.5 log CFU/mL difference) as compared with growth controls run individually. D and D/A demonstrated early and significant bactercidal activity (BC) (99.9% kill) at 2 h against both isolates and A demonstrated BC activity at 32 h vs. MRSA. D/A vs. MRSA was synergistic (>3 log kill) at 48 h and additivity (1–2 log kill) was noted against VREFc. T demonstrated BC activity kill with both isolates at 48 h. L/A demonstrated additivity (1.86 log drop) against MRSA. T/A demonstrated an antagonistic effect against VREFc at 48 h.
Conclusions: MRSA growth is stunted when combined with VREFc in an IVPD model. D and D/A demonstrate early significant BC kill. D/A demonstrate synergy against MRSA and additivity against VREFc at 48 h. L/A demonstrated additivity against MRSA. T demonstrate BC activity vs. both isolates at 48 h, however the addition of A demonstrated antagonistic activity against VREF.

P1043 Pharmacodynamics of amoxicillin/clavulanic acid against *Haemophilus influenzae* in an *in vitro* kinetic model: a comparison of different dosage regimens including a pharmacokinetically enhanced formulation

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Objectives: To compare the pharmacodynamic effects of a new pharmacokinetically enhanced formulation of amoxicillin/clavulanate (A/C) 2000/125 mg (Augmentin XR) twice daily, with A/C 875/125 mg twice daily, 875/125 mg three times daily and 500/125 mg three times daily against *Haemophilus influenzae* with different susceptibility to A/C in an *in vitro* kinetic model.
Methods: β-lactamase producing strains of *H. influenzae* with MICs of 0.5, 1, 2, and 4 mg/L at an initial inoculum of approximately 106 CFU/mL, was exposed to A/C in an *in vitro* kinetic model with a concentration time-profile simulating human serum levels of the new pharmacokinetically enhanced formulation (with a C_{max} of 15 mg/L) twice daily. All isolates were also exposed to A/C with concentration time-profiles correlating to the human dosage of 875/125 mg twice daily (C_{max} 15 mg/L after 1 h), 875/125 mg three times daily and 500/125 mg (C_{max} 8 mg/L after 1 h) three times daily with a simulated half-life of 1 h. Repeated samples were taken regularly during 24 h and viable counts were performed.
Results: A decrease of approximately 2–4 log CFU/mL of all strains was seen after the first exposure of A/C with all four regimens. The initial killing was more pronounced when the initial dose was higher than 500/125 mg. With all regimens against all strains there was a significant reduction in bacterial counts after 24 h as compared with a control. The dosing regimens of 875/125 mg three times daily and the 2000/125 mg XR-formulation given twice daily were able to eradicate the strain with the lowest MIC (0.5 mg/L). For all the other experiments a static effect was achieved at 24 h and there was no significant difference in efficacy between the dosing regimens for the strains with a MIC >0.5 mg/L.
Conclusions: In our experiments T > MIC of 80% was needed to achieve a bactericidal effect (strain with MIC 0.5 mg/L). T > MIC

between 23 and 73% yielded a static effect (strains with MIC 1–4 mg/L) irrespective of the dosing regimen. Our *in vitro* system, like others does not include the potential synergistic effects between the antibiotic and the immune defence factors and may thus reflect the situation in immunocompromised hosts rather than in normal patients.

P1044 The comparative antibacterial effect of ertapenem and ceftriaxone on *E. coli* studied in an *in vitro* pharmacokinetic model of infection

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Objectives: Ertapenem (erta) and ceftriaxone (ceftriax) are once-a-day injectable beta-lactams commonly used to treat Gram-negative infection. There are, as yet, no comparative studies of their antibacterial effects (ABE). We used a pharmacokinetic (pK) model to simulate the serum free drug concentrations of both agents over three doses (72 h) and assess the ABE and emergence of resistance (EoR) for *E. coli*.
Methods: A recent human isolate of *E. coli*, ampicillin susceptible, ceftriax MIC 0.14, erta MIC 0.018 mg/L was used at an inoculum of 106 and 108 CFU/mL. A dilutional, open, single compartment *in vitro* pK model was used to simulate the dosing regimens over 72 h. The pK parameters modelled were: ceftriax 1 g iv 24 hly C_{max} 11 mg/L, $t_{1/2}$ 8 h; ceftriax 2 g iv 24 hly C_{max} 22.0 mg/L; erta 1 g iv 24 hly; C_{max} 13 mg/L, $t_{1/2}$ 4 h. Antibacterial effect was described by reduction in viable count at 24h (delta 24), 48 h (delta 48), 72 h (delta 72), time to clear 99.9% initial inoculum (T99.9) and area-under-bacterial-kill-curve (AUBKC) 0–24 h (AUBKC24), 0–4 8h (AUBKC48), 0–72 h (AUBKC72). EoR was measured by population analysis profiles (PAP).
Results: The ABE (means) were: Ceftriax 1 g was less effective than ceftriax 2 g. Inoculum had an impact on ABE of both erta and ceftriax; for ceftriax regrowth occurred to a greater degree with the high inoculum; for erta regrowth only occurred at the high inoculum. Erta was more effective in the clearance of *E. coli* in the first 24 h compared with ceftriax. No EoR was detected for erta or ceftriax despite the regrowth.

	erta	Ceftriax 1 g	Ceftriax 2 g	erta	Ceftriax 1 g	Ceftriax 2 g
Δ24	−4.3	−4.4	−4.3	−6.03	−2.5	−4.4
Δ48	−4.3	−4.1	−3.2	−2.4	−2.1	−3.6
Δ72	−4.3	−4.4	−3.2	−2.1	−2.0	−3.1
T99.9	3	12	7	4	26	6
AUBKC24	7.3	25.7	20.1	26.1	99.8	60.3
AUBKC48	7.3	27.8	27.0	78.6	182.0	104.2
AUBKC72	7.3	37.6	53.0	165.0	274.1	173.5

Conclusion: Erta produces more rapid clearance of *E. coli* from the model than ceftriax irrespective of inoculum and is less subject to regrowth at an inoculum of 106 CFU/mL. Regrowth is associated with a high initial inoculum for both agents and neither showed any EoR.

P1045 Comparison of ertapenem, ampicillin, and meropenem against the intracellular forms of *Listeria monocytogenes* in human THP-1 macrophages

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Objectives: Ertapenem (ETP) is a new carbapenem with prolonged half-life (approximately 4 h), which could make it more suitable than meropenem (MEM) or ampicillin (AMP) for the treatment of listeriosis. However, *L. monocytogenes* is largely intracellular, and the activity of ETP against these forms has not been investigated. We, therefore, compared ETP to AMP and MPN in a model of human macrophages where AMP and MEM are cidal over a 24 h incubation (approximately 2 log CFU decrease; Carryn *et al.*, *J Antimicrob Chemother* 2003; 51:1051–1052).
Methods: MIC (arithmetic dilutions) and MBC (geometric dilutions) were determined in TSB by standard methods. Activity against extracellular and intracellular forms of *L. monocytogenes* was examined in THP-1 macrophages incubated with extracellular concentrations (ETP, 155 mg/L; AMP and MEM, 50 mg/L) equivalent to the C_{max} achievable in human serum after conventional dosing. The stability of the drugs in the culture medium under our experimental conditions was checked by HPLC.
Results: Activities in broth and in the cellular model are shown in the Table.

	Broth		THP-1 model (change log CFU over 24 h)	
	MIC (mg/L)	MBC (mg/L)	Extracellular (broth)	Intracellular
ETP	0.48 ± 0.03	>64	−0.57 ± 0.05	0.96 ± 0.23
AMP	0.37 ± 0.23	>64	−0.46 ± 0.03	−1.81 ± 0.01
MEM	0.05 ± 0.00	>64	−0.38 ± 0.05	−1.82 ± 0.01

means ± SEM ($n = 3$ independent experiments).

Thus, whereas AMP and ETP have similar activities against *L. monocytogenes* in broth, AMP was cidal but ETP unable to control the growth of intracellular *L. monocytogenes*. Yet, assay of cell-associated ETP showed that its apparent cellular concentration exceeded the MIC. Decreasing the serum concentration in the culture medium (from 10 to 2%) did not change the results. Stability studies failed to reveal significant degradation of extracellular ETP.
Conclusions: In this model, ETP did not eradicate intraphagocytic *L. monocytogenes*. Based on the results obtained MEM, ETP lack of activity is unlikely to be due to its penem structure. It is possible that intracellular conditions (e.g. binding to cytoplasmic proteins) hinder ETP intracellular activity in comparison with AMP or MEM.

P1046 Pharmacokinetic–pharmacodynamic modelling of activities of cefpodoxime and cefixime based on *in vivo* tissue concentrations and *in vitro* kill curves

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The bacterial time-kill curves of cefpodoxime and cefixime against four bacterial strains (*Haemophilus influenzae*, *Moraxella catarrhalis*, *Streptococcus pneumoniae*/penicillin sensitive, and *Streptococcus pneumoniae*/penicillin intermediate) were compared in *in vitro* infection models in which various human pharmacokinetic profiles of unbound antibiotic were simulated. This approach offers more detailed information than the minimum inhibitory concentration (MIC) does about the time course of antibacterial activity of an antibiotic. A pharmacokinetic–pharmacodynamic (PK–PD) model based on unbound antibiotic concentrations at the site of infection and a sigmoid Emax-relationship with EC50 as the antibiotic concentration necessary to produce 50% of the maximum effect effectively described the antimicrobial activity of both cefpodoxime and cefixime. The EC50 values of cefpodoxime and cefixime were consistent with their respective MIC values. Both antibiotics had similar high potency against *H. influenzae* (EC50: 0.04 mg/L) and *M. catarrhalis* (EC50: 0.12 mg/L), while the potency of cefpodoxime against *S. pneumoniae* strains was about 10-fold higher than that of cefixime (EC50s/sensitive strain: 0.02 vs. 0.27 mg/L; EC50s/intermediate strain: 0.09 vs. 0.69 mg/L).

Applications of this model to unbound tissue profiles obtained by microdialysis in a clinical study performed in our group showed that cefpodoxime has higher bacteriological potency against *S. pneumoniae* than cefixime. The developed mathematical PK–PD model (sigmoid Emax model combined with a PK model using free tissue levels) allows for rational antibiotic dosing decisions by predicting the antibacterial effect of various dosing regimens, taking into account the clinically effective free concentrations at the target site. This PK–PD approach is a major improvement over the currently used PK–PD approaches that are usually based on the comparisons of pharmacokinetics of total plasma concentrations and *in vitro* MIC.

P1047 Single-dose pharmacokinetics of cefodizime in acutely ill elderly patients

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Objectives: Cefodizime is an extended-spectrum third-generation cephalosporin antibiotic with good *in vivo* and *in vitro* activity against Gram-positive and Gram-negative pathogens including most beta-lactamase-producing species.
Methods: Pharmacokinetic characteristics of cefodizime were assessed in 21 acutely ill elderly patients (age >65 years). Thirteen patients received a single-dose of 2 g cefodizime intravenously, eight patients received a single-dose of 4 g cefodizime intravenously. Serum concentrations of cefodizime were assessed by high-performance liquid chromatography.
Results: After a single dose of 2 g cefodizime the mean cefodizime serum concentration peak was 222 ± 55 μg/mL, the elimination half-life was 6.19 ± 2.45 h. The total clearance, area under the curve and volume of distribution were 35.8 ± 13.2 mL/min, 1089.4 ± 505.3 μg/mL h and 18.1 ± 6.3 L, respectively. After a single-dose of 4 g cefodizime the mean serum concentration peak was 319 ± 79 μg/mL, the elimination half-life time was 6.81 ± 4.30 h. The total clearance, area under the curve and volume of distribution were 42.35 ± 14.67 mL/min, 1763.2 ± 630 μg/mL h and 21.87 ± 8.08 L, respectively. Cefodizime was tolerated well in all patients, no major side effects occurred.
Conclusion: Our results indicate that acutely ill elderly patients can be treated safely and effectively with a standard dose of 2–4 g cefodizime. No age-related dose-modification is necessary.

P1048 Comparative effects of ertapenem and ceftriaxone on the normal intestinal microflora

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Objectives: Ertapenem is a novel, long-acting parenteral 1-beta-methyl-carbapenem, which is used for treatment of serious infections. Administration of antimicrobial agents causes disturbances in the ecological balance between host and microorganisms. To what extent disturbances occur depends on the spectrum of the agent, the dose, the route of administration, pharmacokinetic and pharmacodynamic properties, and *in vivo* inactivation of the agent. The aim of the study was to investigate the ecological effects of ertapenem compared with those of ceftriaxone on the intestinal human microflora in healthy volunteers.
Methods: Ten healthy volunteers (five females and five males), age range 18–40 years, participated in the investigation. The trial was divided into two 35-day periods. The two treatment regimens were (i) 1 g ertapenem intravenously o.d. for 7 days, and (ii) 2 g ceftriaxone intravenously o.d. for 7 days. Each volunteer received firstly one treatment regimen and secondly the other treatment regimen. The wash-out period was 4 weeks between the two regimens. Faecal samples were collected for microbiological analysis before, during and after treatment.
Results: The number of enterococci increased significantly during the administration of ertapenem, while *E. coli* decreased. There was an overgrowth of low levels of yeasts on day 8. The aerobic microflora was normalised on day 35. Bifidobacteria and bacteroides were markedly reduced while there were minor alterations in the number of lactobacilli and clostridia. On day 35 the anaerobic microflora had returned to normal levels. The faecal concentrations of ertapenem (mean value) were 37.2 and 32.7 mg/kg on days 4 and 8, respectively. *C. difficile* was isolated from four volunteers. Enterococci increased markedly while the number of *E. coli* decreased during the administration of ceftriaxone. No significant overgrowth of yeasts was noticed on day 8. The aerobic microflora had returned to pre-treatment levels on day 35. Lactobacilli, bifidobacteria, clostridia and bacteroides were reduced in significant numbers during the ceftriaxone administration. *C. difficile* was isolated from three volunteers. On day 35 the anaerobic microflora was normalised. The faecal concentrations of ceftriaxone (mean value) were 153 and 258 mg/kg on days 4 and 8, respectively.
Conclusions: Ceftriaxone caused significant ecological changes in the intestinal microflora while ertapenem caused moderate alterations.

P1049 Multiple dose and steady-state pharmacokinetics of ertapenem compared with ceftriaxone in healthy young male and female volunteers

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Background: Ertapenem (ERT) is a new long-acting carbapenem antibiotic with antibacterial activities similar to ceftriaxone. Both drugs have to be administered i.v. once daily.
Methods: ERT (1 g, 30 min i.v.) and CFT (2 g, 30 min i.v.) both once daily for 7 days were compared in a two-way randomised crossover study with 10 (five males and five females) healthy volunteers (age: 35 ± 5 years, weight: 70 ± 13 kg, height: 173 ± 9 cm, crea. clear. 105.4 ± 9.8 mL/min/1.73 m^2, mean ± SD). ERT and ceftriaxone (CFT) were determined via validated bioassay and ERT was additionally determined by chromatography (LCMS). Single dose (SD) and steady-state (SS) pharmacokinetics (PK) were determined by non-compartmental analysis. Statistical comparisons for day 1 vs. day 7 and gender-effects were performed by ANOVA and equivalence statistics.
Results: Values are means ± SD (PE: Point Estimate for ratio SS/SD, CI: 90% confidence interval).

	AUC (μg × h/mL)	Cmax (μg/mL)	$t_{1/2}$ (h)	CLtot (mL/min)
ERT SD,day 1	830 ± 164	256 ± 38	4.6 ± 1.1	20.7 ± 3.7
ERT SS, day 7	836 ± 157	280 ± 52	4.3 ± 0.5	20.5 ± 3.3
ERT PE(CI)	1.01 (0.88–1.16)	1.09 (0.95–1.24)	0.94 (0.83–1.08)	0.99 (0.86–1.14)
CFT SD, day 1	1576 ± 240	315 ± 82	7.6 ± 1.3	21.7 ± 3.9
CFT SS, day 7	1487 ± 227	303 ± 47	7.1 ± 0.9	23.1 ± 4.2
CFT PE, (CI)	0.94 (0.83–1.07)	0.98 (0.83–1.15)	0.95 (0.84–1.06)	1.06 (0.94–1.21)

Urinary recovery was 48 ± 17% (SD) and 44 ± 18% (SS) for ERT. Females (f) and males (m) differed significantly in AUC for ERT ($P < 0.004$; SD: f 711 ± 45, m 950 ± 151; SS: f 770 ± 88, m 901 ± 192 μg h/mL), but not for CFT. The most frequently reported side effect for both drugs was diarrhoea (CFT and ERT: 6/10). No severe adverse events were recorded.
Conclusions: No accumulation in SS was detected for ERT or CFT. Females exhibited a decreased AUC for ERT. However this gender-effect is not considered to be of clinical importance.

Pathogenesis

P1050 Bacterial inhibition of phosphatidylcholine synthesis induces apoptosis in brain cells

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Objectives: *Streptococcus pneumoniae* is the most common cause of bacterial meningitis of high mortality and morbidity. In humans, neurons of the hippocampus undergo apoptosis as a result of meningitis. Phosphatidylcholine (PtdCho) is an essential component of mammalian cell membranes and phosphatidylcholine deficiency, either due to chemicals or altered nutrition, leads to apoptosis, especially in hippocampal neurons.
Methods: Microglia, neurons, and brain endothelial cells were infected with 10^7/mL pneumococcal strain D39 capsular type 2, its unencapsulated derivate R6, the pneumolysin-negative mutant plan−, the pyruvate oxidase mutant spxB−, or the plan−/spxB double mutant for 4 h. Translocation of phosphatidylserine and decrease of mitochondrial transmembrane potential as measurements of apoptosis were detected by FACS analysis. Synthesis of PtdCho was measured by incorporation of [methyl-3H]choline. Inhibition of apoptosis *in vivo* was demonstrated by pretreatment of infected C57BL/6 mice with citicoline. Damage in the hippocampus was quanitified by Tunel staining.
Results: D39 and R6 significantly inhibited phosphatidylcholine biosynthesis (41.2 ± 6 and 67.4 ± 8%, respectively) causing apoptosis of several different types brain cells. Loss of H_2O_2 or/and pneumolysin significantly reduced the ability of pneumocci to cause cell death and caused less inhibition of PtdCho synthesis, with [methyl-3H]choline incorporation into PtdCho increasing to 57.8, 77.9, 92.4%, respectively, compared with uninfected control cells. Supplementation with exogenous lysophosphatidylcholine prevented cell death *in vitro* and treatment of mice with CDP-choline attenuated hippocampal damage during meningitis. Apoptosis inhibitors ZVAD, ALLN, fumonisin B1 or BAPTA-AM did not prevent the bacterial-dependent inhibition of phosphatidylcholine biosynthesis.
Conclusions: We conclude that bacterial inhibition of phosphatidylcholine biosynthesis activates an apoptotic cascade that is a causative event in pathogenesis and is amenable to therapeutic intervention.

P1051 Bacterial cell-wall derived products induce a pro-inflammatory and pro-fibrogenetic phenotype in murine hepatic stellate cells

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Although activation of hepatic stellate cells (HSC) is recognised as the initiating event leading to fibrosis, the factor(s) responsible for HSC activation in non-alcoholic steatohepatitis (NASH) are not completely known. Since patients with NASH have a high prevalence of small intestinal bacterial overgrowth we tested the hypothesis that gut-derived bacterial products may activate HSC. We found by LAL test that endotoxin level (LPS) in portal blood of obese C57lep−/− mice was significantly increased as compared with lean controls (8.74 + 1.08 vs. 6.11 + 0.8 EU/mL). Since we also observed that murine HSC express specific mRNA transcripts encoding mCD14, TLR4, MD2, TLR2 (endotoxin receptors) and peptidoglycan recognition proteins (PGRP), we next studied the effects of LPS, lipoteichoic acid (LTA) or muramic acid (NAM) (10–0.01 μg/mL) on TGF-beta1, IL-6, PDGF-bb, MCP-1 and fibronectin (mRNA transcripts and peptides). Incubation of HSC for 24 h with LPS (1 μg/mL) increased TGF-beta1 (by 1.8-fold) and IL-6 (by 12.5-fold) mRNA transcripts levels over controls, but had no effect on fibronectin mRNA levels. LTA (10 μg/mL) increased only IL-6 (by 4.9-fold) mRNA transcripts, whereas NAM had no significant effects. Similar results were obtained also by cytokine measurements in the conditioned media. Six days exposure of HSC to 10 ng/mL of LPS alone increased TGF-beta1 and IL-6 mRNA transcripts by 2- and 3.9-fold over controls, respectively, but still had no effect on fibronectin mRNA levels. Similarly, LTA and NAM by themselves significantly increased only IL-6 mRNA transcripts by 2.1- and 1.9-fold, respectively. However, simultaneous exposure of HSC to LPS and LTA (10 ng/mL/6 days) induced a fibrogenic phenotype significantly increasing fibronectin mRNA levels by 1.95-fold over controls. In summary, we report that LPS levels are increased in the portal blood of obese mice and that short-term exposure of HSC to bacterial derived cell wall products can induce a pro-inflammatory phenotype whereas a long-term exposure induces a pro-fibrogenic phenotype. We speculate that in obese patients the intestinal barrier function is compromised causing a rise in LPS levels in the portal blood that in turn may contribute to the activation of HSC thus leading to the development of fibrosis in NASH patients.

P1052 Lipopolysaccharide-induced vascular endothelial growth factor expression

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Objectives: VEGF is a potent angiogenic and vascular permeability factor. Recent studies have shown that the VEGF levels are higher in various cells such as macrophages and smooth muscle cells by LPS stimulation, suggesting its importance in the initiation and development of sepsis. LPS-regulated contractility in lung pericytes may play an important role in mediating pulmonary microvascular fluid haemodynamics during sepsis. We have studies the production of VEGF by rat lung pericyte in response to LPS.
Methods: Using rat lung pericytes as a cellular model, we demonstrated that VEGF mRNA and protein expression stimulated by LPS were changed. Pericytes were stimulated with 0.0001–100 μg/mL LPS for up to 12 h to analyse the levels of VEGF mRNA or secretion of VEGF protein. The quantitation of the mRNA levels such as VEGF, iNOS, or 28S ribosomal RNA was verified by RT-PCR. The concentrations of VEGF protein in the conditioned medium or cell lysates were measured with ELISA. To clarify the mechanism of VEGF expression, VEGF expression was examined using the inhibitors of transcription factors such as NF-kB or p38 MAP kinase. Western blot analysis was performed to identify both active and inactive forms of p38MAP kinase in pericytes.
Results: LPS enhanced VEGF mRNA expression in pericytes in a concentration-dependent manner with the maximal levels after 2 h of stimulation. VEGF protein levels, both in the conditioned medium and cell lysate, increased with the increasing concentration of LPS and reached the maximum at 24–48 h after LPS stimulation. LPS also augmented iNOS expression in lung pericytes significantly within 6 h. However, the induction of iNOS mRNA took place later than the LPS-induced increases in the levels of VEGF mRNA. Inhibition of NF-αB or tyrosine kinase did not suppress the LPS-induced augmentation of VEGF mRNA expression in the lung pericytes, although both of inhibitors markedly inhibited the LPS-induced expression of iNOS mRNA. SB203580, p38 MAP kinase inhibitor repressed the LPS-induced VEGF mRNA expression. LPS stimulated a rapid and sustained phosphorylation of p38 MAP kinase. These results indicate that LPS induce the VEGF expression in lung pericytes through p38 MAP kinase.
Conclusion: We conclude that pericytes produce VEGF in response to the stimulation with LPS, which may be partly mediated by the p38 MAP kinase pathway.

P1053 Antistaphylococcal effect of recombinant human erythropoietin

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Objective: Erythropoietin (EPO) was originally identified as a hormone for adjustment of the circulating erythrocyte mass. Following molecular biologic studies today it is known that EPO is a member of the cytokine superfamily with significant homology to mediators of growth and inflammation. In this study, we questioned the antibacterial effect of EPO against an intracellular pathogen.
Methods: A human isolated, non-mutant, non-37°C resistant *Salmonella typhimurium* strain was used in experiments. Resting mouse peritoneal macrophages were incubated for 24–48 h with EPO (10 U/mL), EPO/2 (5 U/mL), EPO/4 (2.5 U/mL), L-NAME (nitric oxide inhibitor), EPO/L-NAME, EPO/2/L-NAME in 10:1, 5:1, 1:1 m.o.i. Following incubations extracellular nitric oxide (NO) levels (Griess) were determined; apoptosis (Hoechst 33342)/necrosis (propidium iodide) determinations were made. In simultaneous groups, cells were lysed with sterile distilled water and colony counts were performed.
Results: Following 24–48 h colony counts were significantly lower in EPO groups compared with EPO/2, EPO/4, *Salmonella*, L-NAME, EPO/L-NAME groups in 10:1, 5:1, 1:1 m.o.i. Interestingly, NO responses were higher in EPO groups compared with EPO/2, EPO/4, and *Salmonella* groups. In apoptosis/necrosis balance, apoptosis was dominant in EPO groups compared with EPO/2, EPO/4, *Salmonella*, EPO/L-NAME, L-NAME groups.
Conclusion: It is known that EPO is a multifactorial tissue protective cytokine. *S. typhimurium* shows its pathogenicity by entering the cell, and when enters the cell NO response and apoptosis starts. This study showed that EPO inhibited the entry of *S. typhimurium* into the macrophages, and simultaneously stimulated NO response which triggered apoptosis more than the bacteria expected.

P1054 *Ex vivo* release of pro-inflammatory cytokines of blood monocytes of patients with sepsis: correlation to the initiation of symptoms and to monocyte apoptosis

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Objectives: Failure of immunomodulation in sepsis is partly attributed to lack of knowledge for the appropriate time necessary for administration of therapy. This study was designed to correlate the time of the presentation of symptoms to the excretory effect of patients' monocytes in an attempt to identify the appropriate time period for immunomodulatory intervention.
Methods: Heparinised venous blood was sampled from 37 patients with sepsis according to the ASCP/SCCM 1992 criteria. Blood was centrifuged over Ficoll and mononuclears were incubated in RPMI with 10% FCS. After removal of non-adherent cells, monocytes were harvested and half of them were re-suspended in wells with medium without/with the addition of 4% of the serum of the patient and incubated for 18 h. The remaining half of cells was lysed and caspase-3 activity was estimated in the cytosolic extract by a chromogenic assay. Tumour necrosis factor-alpha (TNF) and interleukin-6 (IL-6) were estimated in culture supernatants by EIA; malondialdehyde (MDA) was assessed by the thiobarbiturate assay. Results were correlated to the time lapsed since advent of fever.
Results: Median TNF excreted from monocytes of patients with fever within 12 h, 13–24 h and more than 24 h post-admission were 43.8, 21.9 and 29.6 pg/10 000 cells, respectively, and became 23.4, 21.1, 10.5 pg/10 000 cells, respectively, after incubation with the patients' serum. Median IL-6 excreted from monocytes of patients at the above time intervals were 118.1, 58.1 and 32.8 pg/10 000 cells, respectively, and became 163.2, 48.7, 52.6 pg/10 000 cells, respectively, after incubation with the patients' serum. Median MDA excreted from monocytes of patients at the above time intervals were 1.52, 0.67 and 2.43 mmol/10 000 cells, respectively, and became 2.11, 1.06, 2.02 mmol/10 000 cells, respectively, after incubation with the patients' serum. Median monocyte caspase-3 activities at the above time intervals were 53.4, 200.0 and 99.7 pmol/min/10 000 cells.
Conclusions: Elevated biosynthetic activity of human monocytes is found over the first 12 h of symptoms of sepsis. Latter advent of symptoms is accompanied by lower cytokine release and higher apoptotic activity leading to the assumption that immunomodulatory intervention in sepsis should be kept only for patients with early occurrence of symptoms.

P1055 Adherence factors of ocular isolates of nontypeable *Haemophilus influenzae* from Saudi Arabia

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Objectives: Nontypeable *Haemophilus influenzae* is a common cause of otitis media, sinusitis and respiratory infections. In some countries it is also a significant cause of conjunctivitis. The adherence of *H. influenzae* to epithelial cells is an initial step in its pathogenesis. Such adhesion is not thought to be mediated by fimbriae, but several non-fimbrial adhesins have been identified. HMW1 and HMW2 are related high molecular weight adhesins, present in many strains. Some strains express an alternative adhesin, Hia, instead. Most of the studies examining the types and distribution of these factors have looked at respiratory and otitis media isolates collected in Western countries. This study investigated the type and prevalence of adherence factors in ocular isolates from Saudi Arabia.
Methods: Isolates were collected from patients in Riyadh presenting with ocular infections (OI, 100 isolates) or respiratory infection (RI, 19). Commensal isolates were obtained from the eyes of healthy volunteers (CE, 18 isolates). Strains were identified by standard methods and biotyped by API NH strips. PCR was used for serotyping and detection of the genes encoding HMW1, HMW2, and Hia.
Results: All 137 isolates were confirmed as nontypeable, and none produced a PCR amplimer with primers designed against the published sequence of hmw1. The prevalence of the hmw2 gene amongst the different groups was: OI 47% +ve, RI 37% +ve, and CE 39% +ve. The proportion of strains with hia was OI 46%, RI 21%, and CE 0%. Strains carrying hmw2 and hia together were identified amongst the ocular infection and respiratory infection groups (20 and 16% of isolates, respectively). Such strains were spread across biotypes 2, 3, and 4. The percentage of strains with neither adhesin was lower in the ocular infection group (27%) than in the RI (58%) and CE (61%) groups.
Conclusion: Previous studies based on non-ocular isolates have suggested the HMW and Hia adhesins are not found together in strains. In this study, a significant proportion of nontypeable *H. influenzae* isolates from ocular and respiratory infections were positive for both. The mixture of biotypes suggests such strains were not members of a clonal group. The gene encoding HMW1 was not detected amongst the 137 isolates. The distribution of HMW and Hia adhesins amongst isolates causing disease in Saudi Arabia appears to differ from those isolated in Western countries.

P1056 Genetic relationships among nontypable *Haemophilus influenzae* (NTHI) isolates presenting different adhesins profiles

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Objective: Nontypable *H. influenzae* (NTHI) commonly colonising human nasopharynx and causing the localised respiratory tract

infections are known to demonstrate substantial strain heterogeneity. Within NTHI group, strains of different combinations of particular adherence factors (e.g. HMW1/HMW2 family like proteins, Hia, Hap and pili) might be capable to different extent colonise the host and induce infection. Comparison of the adherence patterns seen in NTHI isolated from carriers and from localised respiratory tract infections and their fingerprinting profiles might help to determine some relationships among epidemiologically unrelated strains/clades. Those relationships might suggest different level of invasive potential.
Methods: We studied 55 NTHI isolates obtained from patients with localised respiratory tract infections. Randomly amplified polymorphic DNA (RAPD) and automated amplified-fragment length polymorphism (AFLP) techniques with fluorescently labelled primers were used to fingerprint NTHI isolates. PCR has been used to evaluate gene organisation both of the pilus (hif) (detection of insert within the purE-pepN region) and hmw clusters.
Results: Among 55 NTHI strains isolated from patients with bronchitis, pneumonia, pharyngitis and otitis, three different arrangements of hif locus were found. For 14 strains 0.3 kb fragments indicating lack of insert hif were found. The remaining 41 strains harboured fragments of intermediate length, ranging from 0.9 to 1.1 kb. Locus of 3.4 kb, commonly seen in strains isolated from healthy carriers, was not identified in any strain tested. Genes encoding HMW1/HMW2 like proteins were found to predominate among isolates associated with pneumonia and pharyngitis diseases. Most of the strains harboured hmw+ locus and genotype of 0.9–1.1 kb hif locus. Only three hmw+ strains were found not to contain insert hif. Genotypes of the purE-pepN region were grouped into relationship classes by AFLP and RAPD and hmw patterns. All genotypes grouped within a single AFLP set had identical or near identical hmw patterns.
Conclusions: Relationships among strains classified according to perE-pepN and hmw genotype, and AFLP and RAPD dendrograms clusters were found.

P1057 Bac protein based recombinant peptides as possible means for vaccine development against GBS infection

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Objectives: Group B streptococci (GBS) being the main cause of infection pathology of the newborns can also cause serious infections in adults. Construction of the recombinant vaccine against GBS infection might be important for the several risk groups including pregnant women. Several recombinant peptides based on the GBS IgA binding protein Bac were used for vaccination of mice in order to evaluate their immunogenicity and possible protection against GBS infection.
Materials and methods: GBS strain 219, type IIbc was used as source of DNA for cloning. Bac gene fragments corresponding to the 3′ portion of the gene were obtained by PCR and cloned in *E. coli* expression vectors. Recombinant proteins P1, P5, P6 (26, 24, 35 kD) were purified by affinity chromatography and used for subcutaneous immunisation of mice. Immunisation was accomplished in three doses of the antigens (5, 10 and 20 μg) two times each dose with interval of 4 weeks. ELISA monitored antibody titres starting from the 21st day until the 150th day from the beginning of immunisation. Constant of association (K_a) of the antibodies was estimated employing computer software package 'Polyconst'. Protection studies were accomplished in mice infected intraperitoneum by GBS with doses 106 and 107 cells per mice.
Results: Three Bac based recombinant peptides were used for immunisation of mice. Largest peptide P6 generated the highest titres of antibodies (1×106) on the 69th day of experiment. That titre was not changed till the end of the study. K_a of the antibodies to the P6 peptide varied from 108 to 1013 M-1 with the 50% share of high affinity IgG. Immunisation with peptides P1 and P5 reached maximum on the 41st or 48th day of the experiment with titres 1:50 000 or 1:12 000, respectively, with gradual decrease till the end of the study. Affinity of IgG to those peptides also varied between 106 and 1012 M-1. Share of high affinity IgG was 10–30%. Cross-absorbtion of the antibodies to the peptides demonstrated the homology between anti-P6 and anti-P1 and differences with anti-P5. The most immunogenic peptide P6 was used for mice protection study. It was shown that mice with IgG titre against P6 equal to 1:100 000 were protected against GBS infection and GBS was eliminated from blood and peritoneum.
Conclusion: Bac based peptides P6 and P1 can be considered as possible vaccine components against GBS infection. Perspectives of GBS vaccine development and epitop organisation of Bac proteins are discussed.

P1058

Abstract withdrawn.

P1059

Abstract withdrawn.

P1060 Bacterial adherence ability and adherence factors of quinolone sensitive and resistant *E. coli* strains isolated from cirrhotic patients

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Objectives: Prolonged use of norfloxacin as secondary prophylaxis of spontaneous bacterial peritonitis entails an appearance of 40% of *E. coli* quinolone-resistant (QR) in stools. However, the development of infections caused by these QR bacteria is not so frequent as expected. Our aim was to assess bacterial adherence (BA) of either quinolone-sensitive (QS) or QR strains to oral epithelial cells in presence/absence of sub-MIC concentrations of norfloxacin and to evaluate the influence of some relevant adherence factors presence on adherence to cells.
Methods: 59 strains of *E. coli* obtained from rectal swabs of cirrhotic patients were studied and their MIC was determined by E-test. Twenty strains from healthy volunteers were also tested as controls. Strains were incubated with oral epithelial cells in presence/absence of sub-MIC of norfloxacin. BA was measured by the percentage of epithelial cells with attached bacteria (marked with fluorescein). Adherence factors were determined by PCR of specific sequences for type I fimbriae, intimin and afimbrial adhesin. Type I fimbriae expression was also measured by agglutination inhibition with manose. Statistical analysis was performed with the SPSS package. Student's *t*-test or *U*-Mann–Whitney test was applied for quantitative variables, and Ji-Square test or Exact Fisher test were applied for qualitative ones.
Results: 22 QR and 37 QS strains were isolated. BA was similar in both series (78.2 vs. 80.6%, P = NS), and these percentages were similarly and significantly reduced when norfloxacin was added (48.5 vs. 45.0%, P = NS) ($P < 0.001$). The most frequent factor was type I fimbriae (71%), whose expression was shown in 52.3%. Eighteen per cent of the strains lacked the adherence factors studied. Adherence factors presence was less frequent in QR strains

than in QS. Adherence observed is independent of the presence/absence of the adherence factor studied. The joint presence of various adherence factors is correlated with a slightly increase in BA, independently of the sensitivity to quinolones. No differences were found respect to the control strains results.
Conclusion: BA capacity of *E. coli* is independent of its sensitivity to quinolones, although QR strains seemed to be less infective. Subminimal concentrations of norfloxacin decreases BA in both bacterial phenotypes in the same degree. This result would support the continuous use of norfloxacin in this patients, in spite of the selection of QR strains in stools.

P1061 Effect of quinolones on the induction of the loss of pathogenicity islands (PAIs) in uropathogenic *Escherichia coli*

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Objective: In previous studies, a decrease in the virulence factors was observed in quinolone-resistant vs. quinolone-susceptible uropathogenic *E. coli* strains. The aim of this study was to analyse the possible role of quinolones in the induction of the loss of PAI which contains the hlyA (haemolysin) and cnf1 (cytotoxic necrotising factor) genes in uropathogenic *E. coli*.
Methods: Ten quinolone-susceptible uropathogenic *E. coli* strains containing some PAIs were subjected to subinhibitory concentrations of quinolones: nalidixic acid, ciprofloxacin, moxifloxacin, and levofloxacin; and other antimicrobial agents such as ampicillin and trimethoprim. In each step, the strains were spread on blood agar plates to test the loss of haemolysin capacity. Non-haemolytic colonies were tested by PCR to test the presence of hlyA and cnf1 genes, as well as other virulence factors such as sat, iha, focG, pap genes; and by PFGE, Southern blot and hybridisation with hlyA, cnf1 and sat probes. In parallel, the wild-type strains were subjected to the same procedure with antimicrobial-free culture media, to detect a possible spontaneous loss of PAI. The phylotype was analysed by PCR with specific primers. Hypermutation assays were carried out by spreading of an overnight culture onto MH plates containing rifampicin 50 mg/mL to determine the number of colonies able to grow on these plates.
Results: Non-haemolytic colonies were found in nine strains selected for analysis. These colonies were found in five isolates with nalidixic acid; five with ciprofloxacin, three with levofloxacin; and only in one isolate with moxifloxacin. In most of these isolates, no mutations in the gyrA gene were observed. However, non-haemolytic colonies were not observed when the strain was subcultured in antimicrobial-free culture media. It is noted that only one strain lost the PAI when the strain was submitted to subinhibitory concentrations of ampicillin or trimethoprim. All these colonies were hlyA and cnf1 negative by PCR and by hybridisation of PFGE profiles. The colonies derived for wild-type submitted to subinhibitory concentrations of nalidixic acid, which contain the iha gene, lost this gene. No relationship was found between hypermutation phenotype or phylotype and capacity to lose the PAI.
Conclusions: Subinhibitory concentrations of quinolones may induce the loss of PAIs in some uropathogenic *E. coli* strains. This fact could be due to the activation of the SOS system.

P1062 Use of deletion mutations to explore structure-function relationships in *Clostridium tetani* neurotoxin

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Objectives: *Clostridium tetani* neurotoxin represents one of the most toxic substances known to date. The most effective way to prevent intoxication caused by this molecule includes immunisation of humans with a toxoid. Such vaccines, used currently throughout the world, represent partially purified from *C. tetani* cultures and detoxified by formalin treatment protein. Considerable efforts are being carrying out in order to improve anti-tetanus vaccine using gene-engineering approach. The aim of such studies is to obtain fully immunogenic yet void of toxic activities molecule. However, this goal requires detailed knowledge in toxin's domains, necessary for its lethal activity. One such domain is known to include critical glutamic acid residue-234 (Li *et al.*, 2001). In our study, using deletion mutagenesis approach, we investigate another critical part of tetanus neurotoxin-junction region between light and heavy chains.
Methods and results: Initially, we cloned the gene and produced full-size *C. tetani* neurotoxin, which was lethal for mice by yielding clinical picture of typical tetanus. Then, using PCR strategy, we deleted nucleotide sequences, coding for either amino acid residues (aa) 429–457, 419–428 or 408–418. By studying biological activities of the expressed protein product, we found out that deletion of the last 29 amino acid residues of L chain (aa 429–457) resulted in the non-toxic molecule. According to proteolysis profile, mutated protein preserved overall structure of the active recombinant neurotoxin and thus was speculated to retain its antigenic properties.
Conclusions: In our previous investigations we produced and studied different individual fragments of tetanus toxin (L-chain, H-chain, Hc domain) and two hybrid proteins, one composed of tetanus toxin fragment Hc and C-terminal domain of *C. difficile* toxin B and another made of fragment Hc and S3 domain of *C. histolyticum* collagenase (Varfolomeeva *et al.*, 2003). During the current study we continue this work by constructing a new set of molecules, which could be considered as candidates for testing in vaccine development. On the other hand, data obtained shed some light onto the mechanisms of *C. tetani* neurotoxicity, confirming absolute necessity of intact interchain region for neurotoxin activity.

P1063 The contribution of a subspecies-specific type IV secretion system to pathogenicity in *Campylobacter fetus venerealis*

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Objective: The pathogenicity mechanisms employed by *Campylobacter* species remain poorly understood. Striking host specificity is exemplified by the highly related taxa *C. fetus fetus* (Cff) and *C. fetus venerealis* (Cfv). Cff is transmitted orally and usually colonises the intestinal mucosa of humans and sheep, causing gastroenteritis and septicaemia, whereas Cfv is transmitted venereally and induces epidemic abortion in cattle. The genetic basis for the difference in host and tissue specificity of *C. fetus* subspecies is studied.
Methods: Subtractive hybridisation of the entire genomes of Cff vs. Cfv identified genes unique to Cfv. The DNA was sequenced and specific genes were mutagenised. Phenotypic variation for DNA transfer, adherence and infection were assessed *in vitro*.
Results: A type IV secretion system (T4SS) is exclusively present on the chromosome of Cfv. T4SSs are complex multi-protein systems assembled by pathogenic bacteria to span the cell envelope and to deliver virulence factors to target cells. The unique Cfv DNA spans 30 kb and encodes homologues to mobility genes (phage integrase, IS transposase) and virulence determinants. Moreover, homologues with similar genetic organisation as the *A. tumefaciens* VirB operon including all components from virB3 to virB11 and virD4 were identified. Selected Cfv homologues to genes essential to function in the highly related T4SSs of *A. tumefaciens*, *Helicobacter*, *Brucella*, and *Bartonella* were inactivated through recombination.

Conclusions: The genomes of *C. fetus* subspecies Cff vs. Cfv differ at least by the additional presence of a 30 kb element resembling a pathogenicity island in Cfv. This inserted sequence encodes homologues of genes associated with lateral transmission as well as virulence. Most importantly, it encodes genes phylogenetically related to the VirB-VirD4 operon conserved in a variety of Gram-negative pathogens. Genetic analysis combined with functional *in vitro* assays will determine the relevance of this newly discovered T4SS in *Campylobacter* virulence and its contribution to tissue specificity and host adaptation.

P1064 The role of the SabA adhesin and other *Helicobacter pylori* virulence factors in activation of human neutrophils

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Objectives: A prominent feature of *Helicobacter pylori* (Hp) induced gastritis is an infiltration of neutrophils into the epithelium surface. Some Hp strains (40–60%) have the capacity to non-opsonised activate neutrophils to release reactive oxygen and nitrogen species and proteases, and such strains were more often isolated from patients with peptic ulcer disease. Several soluble factors were described to be involved including urease, HPNAP, a low molecular weight factor in water extract of the bacteria and the cecropin-like bactericidal peptide, but none have stood the test. In the present study we used isogenic knock-out strains to study the role of various Hp virulence factors for the nonopsonic activation of neutrophils.
Materials and methods: Human neutrophils were challenged with wild type (wt) Hp strains and isogenic knock-out mutants deleted of HPNAP, BabA adhesin, SabA adhesin, VacA cytotoxin or the 37 kDa fragment of VacA, followed by chemiluminescence (CL) measurements of the superoxide anions produced by the neutrophils. The effects of signal transduction inhibitors on Hp-induced neutrophil activation were also studied to identify intracellular signalling pathways.
Results: The wt Hp strains induced in neutrophils a strong CL response. The absence of HPNAP or the Leb-binding BabA adhesin had no effect. However, no response was obtained after the deletion of the sialyl Lex-binding adhesin, SabA. Deletion of the whole VacA cytotoxin resulted in a delayed and reduced CL response, and the same effect was observed when the 37 kDa fragment was deleted. Effect of pertussis toxin indicated that the induction is transduced by a member of the G protein receptor family.
Conclusions: The study shows that SabA adhesin plays a pivotal role in the nonopsonic activation of neutrophils and might therefore be an important Hp virulence factor.

P1065 Characterisation of the penetration ability and cytotoxicity of *Serratia marcescens*

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Objectives: To characterise the penetration ability and cytotoxicity of clinical isolates of *Serratia marcescens* that caused either invasive or noninvasive infections.
Methods: Nineteen each of blood and urinary isolates of *S. marcescens* were examined *in vitro* for their ability to penetrate a Madin-Darby canine kidney (MDCK) epithelial cell monolayer by using a membrane filter system. The cytotoxicity against MDCK cells was assessed by measuring the concentration of lactose dehydrogenase (LDH) released from the cells into the medium. The Chi-square test was used for statistical analysis.
Results: Significantly more blood than urinary isolates were detected in the basolateral medium after 6 h (18 vs. 10, $P < 0.01$) incubation. Nine blood isolates compared with only two urinary isolates were high penetrators (>10 000 000 CFU/mL, $P < 0.05$). A similar significant difference in cytotoxicity between the two groups was also found (17 blood vs. 9 urinary, $P < 0.05$). The average LDH level of all blood isolates at 6 h after inoculation was 157 ± 74 U/L (median, 168 U/L) and that of all urinary isolates was 92 ± 63 U/L (median, 58 U/L). LDH level increased proportionally as the number of bacteria that penetrated increased, indicating a close association of the penetrative ability of bacteria to their cytotoxicity. By using a fluorescent acridine orange-crystal violet staining method, viable bacteria were observed intracellularly at 2 h, and the amount increased at 3 h, indicating the invasive ability of *S. marcescens* and a possible mechanism of transcytosis penetration. No apparent correlation between genotypes and the ability to express penetration and/or cytotoxicity. Neither plasmid profiles nor antimicrobial resistance patterns were correlated with the ability to penetrate or to cause cytotoxic effect, indicating that the genes encoding the associated factors are not located on the plasmids. Further characterisation of the cytotoxicity indicated that viable whole bacteria were required to induce cytotoxicity *in vitro*, and neither toxic exoproducts produced during growth nor intracellular materials were responsible for the cytotoxic effect. Inhibition of RNA and protein synthesis abolished the cytotoxicity as well as the penetration.
Conclusions: Although other mechanisms may be involved, the abilities to penetrate epithelial barriers and the associated cytotoxicity are important virulence factors contributing to invasive infections associated with *S. marcescens*.

P1066 Modulation of *Listeria monocytogenes* interaction with cellular substrate by β-lactam antibiotics and human serum transferrin

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Background: Interactions between *L. monocytogenes* and cellular substrate represents a very important model for the study of molecular mechanisms in intracellular parasitism. *L. monocytogenes* harbours many virulence factors which expression is regulated by iron(III) concentration. This bacterium owns three different iron uptake systems, one of them involving probably a bacterial cell surface-located transferrin-binding protein.
Purpose: we have investigated how interaction between *L. monocytogenes* and cellular substrate is modulated by β-lactams and human serum transferrin (HST) and the effect of HST on the expression of listerial virulence factors.
Material and methods: The *in vitro* study of adherence and invasion capacity of six *L. monocytogenes* strains to cellular substrate represented by Hep-2 cells was investigated by gentamycin-protection assay. The ability to adhere to an inert substrate was evaluated by the slime test. The phenotypic detection of virulence factors represented by Kanagawa haemolysin, sheep erythrocytes haemolysins, DN-ase, lipase, lecitinase, amylase, gelatinase and caseinase was performed on specific media. The influence of transferrin on bacterial growth was evaluated by determining the number of CFU.
Results: β-lactam antibiotics and vancomycin in subinhibitory concentrations modulate the adherence pattern from a normally diffused-one to a localised-one. Transferrin has a general stimulatory effect on *L. monocytogenes* growth rate and cell surface molecules expression demonstrated by: (1) greater adherence capacity to the inert surfaces; (2) a higher number of CFU/mL and (3) an increased production of all tested virulence factors with the exception of Kanagawa toxin and DN-ase in the presence of transferrin.
Conclusions: We conclude that beta-lactams probably have affected the bacterial adherence pattern to the cellular substrate by inducing changes in the negative charges on bacterial surface. The increase in cell surface hydrophobicity decreases the electrostatic repulsion between cells, stimulating the clustered-adherence pattern. These aspects were also correlated with the results of slime test. We also have demonstrated that transferrin has stimulated the bacterial growth rate and the production of virulence factors probably by inducing the expression of genes coding for these virulence factors.

P1067 *Treponema pallidum pallidum* (strain Nichols), but not *Treponema phagedenis* (biotype Reiter), has an apical structure that may be a pathogenic factor

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Objectives: The re-emergence of syphilis in AIDS time justifies the return to the investigation about *Treponema pallidum* underestimated by the successive failures on trying the culture of this bacterium in continuous cell line.
Methods: Morphological studies on *T. pallidum pallidum* (strain Nichols) maintained in rabbit testis and *T. phagedenis*, biotype Reiter, growing in Spirolate Broth supplement with chicken serum, were visualised by Transmission Electron Microscopy in ultra-fine sections with negative staining techniques with ammonium molybdato, with and without chemical dissection by non-ionic (Triton x100) and anionic (SDS) detergents in different concentrations.
Results: Beyond confirming the close relation between the peptidoglican (P) and the cytoplasmic membrane (CM), the fragility of the outer membrane (OM) that surrounds the periplasmic flagella (PF) which possess Gram-positive basal plates, and also the existence of cytoplasmic filaments (CF), it allowed: (i) to observe ultrafine sections of the cytoplasmic insertion of the basal plates of PF doing what Hovind and Hougen predicted 30 years ago with negative stain technique; (ii) to see that the basal apparatus is complex, involved by the 'mot proteins' that give them the shape of a sphere inserted in the cytoplasm, after the flagellum hook has passed the P and CM; (iii) from this motor apparatus have their origin the CF, which extend along the cytoplasm, accompanying the PF that are present in a greater number; (iv) in some pictures from cells of *T. pallidum* treated with SDS, a membrane is noted inside these filaments, suggesting a close relation between them and the flagella; (v) in *T. pallidum*, but not in *T. phagedenis*, is observed, in the extremity, an apical structure that may be related to the fixation of these pathogenic treponema to the host cells.
Conclusions: The assumption that this apical structure is an important factor of pathogenicity is reinforced by being exclusive of *T. pallidum*, the observation of adherence by one of the extremities to testicular cells, when we extract these from rabbit testis; and in the cases were the culture in cotton-tail testicular cells show some success the Treponemas were adhere to the cells. These observations emphasise the importance to proceed the investigation about these fascinating bacteria, trying to properly elucidate this apical structure.

P1068 Route of natural exposure in animal model of brucellosis

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Objectives: The aim of this study is experiencing the route of natural exposure instead inoculation of the bacteria intraperitoneally as in the existent animal model of brucellosis. Consequently, improve the model's reliability and extension to the new areas may provide new insights into the understanding of human brucellosis.
Methods: In this study, adult Wistar rats weighing 150–180 g were used. The rats were matched by weight and paired so that one rat of the pair was assigned as vector (*n*: 9) while the second rat was target (*n*: 9). Target rats were marked with nail polish. Paired rats were then caged individually. All vector rats in each cage were inoculated with *B. melitensis* intraperitoneally. Cells of *B. melitensis* strain 16 M were suspended in a saline and suspensions were adjusted to yield 2×10^4 to 4×10^4 CFU. Inoculation was performed by injecting one dose of 0.5 mL saline containing 2×10^5 to 4×10^5 intraperitoneally to vector rats. Seven days after challenge to *B. melitensis*, all rats were weighted and assessed for the number of *B. melitensis* isolated from spleens. Spleens were aseptically removed, weighted and a piece of each organ was homogenised in 1.0 mL of sterile saline. Aliquots of 0.1 mL of the homogenates were diluted tenfold in saline plated on to Brucella agar plates to obtain a viable count. Comparative analysis between groups, of mean CFU/organ weight, rat body weight, spleen weight and their ratio were carried out by using the Mann–Whitney test. A P value of <0.05 was considered to be statistically significant.
Results: The number of *B. melitensis* isolated (CFU) from spleen in vector rats was 1048.0 ± 52.80, 869.0, 1367.0 (Mean $\pm$ SEM, min, max). All the target rats (100%) were found to be infected with *B. melitensis*. The target rats received the infection from the vector rats and the number of *B. melitensis* isolated (CFU) from their spleen was 148.1 ± 24.81, 62.67, 255.7 (Mean $\pm$ SEM, min, max). The number of *B. melitensis* isolated from spleen in the vector rats ($P < 0.05$) was significantly greater than the number in the target rats.
Conclusion: In this study, the model of *B. melitensis* infection in the setting of inoculation via route of natural exposure was modified and it was observed that the natural route of inoculation is as affective as the route of intraperitoneal inoculation. Further use of this model may provide new insights into the therapy of humans who may be threatened with this organism through zoonotic infections and potentially bio-terrorism.

Insights into Hepatitis

P1069 Efficacy of lamivudine in treatment of precore mutant chronic hepatitis B

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Objectives: Precore mutant chronic hepatitis B is very common in Mediterranean countries, and these patients are at risk for developing of cirrhosis. This study was conducted to evaluate the efficacy of one year of lamivudine therapy in these patients.
Methods: From April 2000 to January 2003, 41 consecutive patients with chronic anti-HBe positive, HBV DNA positive hepatitis B were treated with 100 mg lamivudine daily for 1 year, in Babol, Iran. Alanine aminotransferase (ALT) levels, HBV DNA, histological activity index (HAI) score and fibrosis score at the baseline and the end of treatment were compared by chi-square and Fisher's exact tests.
Results: 41 cases (38 males, 3 females) with the mean age of 32.4 ± 11 years were treated. At the baseline, mean ALT levels were 91 ± 35 IU/L and mean HAI score was 5.5 ± 3. After treatment, HBV DNA became negative in 20 (48.8%) of cases. Histological activity index score was improved by two points or more in 10 (24.4%) and increased in 13 (31.7%) of the cases. ALT returned to normal values in 30 (73.2%) cases. HBV DNA became negative in 12 (60%) cases with ALT levels of more than 80 IU/L, but eight (40%) in cases with ALT levels of less than 80 IU/L

($P = 0.57$). Reduction of fibrosis score was seen in one (6.3%) case with ALT levels more than 80 IU/L, compared with three (12%) in cases with ALT levels less than 80 IU/L. Reduction of HAI score was seen in four (25%) cases with ALT levels more than 80 IU/L, but in six (24%) cases with ALT levels less than 80 IU/L ($P = 0.93$). No patients lost HBsAg.
Conclusion: The results show that 1 year of lamivudine therapy is not effective in most patients with precore mutant chronic hepatitis B. Longer duration of therapy is necessary for these group of patients.

P1070 Molecular epidemiology of hepatitis B in Denmark

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Objectives: Denmark has a low incidence of acute hepatitis B (HBV) infections but the impact of an increasing number of immigrants with chronic HBV infection on HBV transmission is unknown. The aim of this study was to characterise individuals with chronic and acute HBV infection in a defined region and to examine relations between them.
Methods: During 2000–2001 all consecutive HBV infected individuals in the County of Funen, Denmark, were classified according to ethnicity, presumed route of transmission and stage of infection. HBV DNA was sequenced and subjected to phylogenetic analysis.
Results: Of 309 identified HBV infected individuals, 218 (71%) were classified as having chronic infection and 91 (29%) as having acute infection. HBV DNA sequencing was possible in 125 cases. Phylogenetic analysis showed that HBV isolated from injecting drug users (IDUs) were identical or closely related. In other chronic cases, the viral geno- and subtype mainly reflected the individual's ethnic origin. Among acute cases acquired in Denmark, 89% (74/83) were related to IDU (65 cases in IDUs and nine cases in individuals presumably exposed to IDUs). Among 83 ethnic Danes who acquired their HBV infection in Denmark, no new cases of transmission from immigrants to ethnic Danes were detected.
Conclusion: Injecting drug use was the single most important factor for hepatitis B transmission in Denmark. The current Danish vaccination strategy is unable to protect IDUs from HBV infection and IDUs pose a greater risk of HBV transmission to the general population than immigrants. With few exceptions chronic infection reflects a history of IDU or foreign ethnic background.

P1071 Much gained by integrating contact tracing and vaccination in the hepatitis B antenatal screening programme in Amsterdam, 1992–1999

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Objectives: Hepatitis B control in Europe concentrates on antenatal screening to reduce vertical transmission. To reduce horizontal transmission and the pool of infectious individuals, the Municipal Health Service of Amsterdam integrated tracing and immunising of contacts in the antenatal screening programme.
Methods: An 8-year (1992–1999) descriptive study of this public health programme, where contacts are tested for serological markers of previous infection, and vaccination is offered to susceptible contacts. Chronically infected contacts are counselled and referred for treatment if justified.
Results: For 738 newly identified women testing positive for the hepatitis B surface antigen, 1219 contacts were reported; 1100 (90.4%) contacts participated, 476 (43%) had serological markers of previous infection, of whom 119 (25%) were infectious. Of the 603 eligible contacts, 568 (94%) completed the vaccination series. Country of origin was an independent predictor of contact participation and compliance with completion of the vaccination series. Postvaccination titres for antibodies against the surface antigen were below 10 IU/L in 4.5% of contacts under 30, in 12.2% of those over 30.
Conclusions: Tracing and immunising susceptible contacts of women screened as HBsAg-positive should be an integral component of any nations HBV control programme.

P1072 Different B2-microglobulin (b2m) curves in chronic hepatitis B (CHB) patients, under long-term lamivudine (LAM) monotherapy or interferon plus lamivudine (IFN/LAM) combination treatment. Relationship with virological breakthrough (VB)

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Objective: To evaluate the predictive value of serum b2m levels for VB, in CHB patients under long-term LAM monotherapy or initial combination (IFN/LAM) treatment.
Methods: Serum b2m levels were calculated at baseline and every 3 months during treatment, in 25 HbeAg-negative CHB patients under long-term (36 months) LAM monotherapy (group A) and 12 patients under IFN/LAM treatment for the first 6 months followed by LAM monotherapy thereafter (group B), using the microparticle enzyme immunoassay technology. We used Cox proportional hazard models in order to investigate the association between serum b2m levels and VB.
Results: Seven of 25 patients (28%), 9/25 (36%) and 14/25 (56%) from group A and 0/12, 2/12 (16.6%) and 3/12 (25%) from group B exhibited VB at months 12, 24 and 36 of treatment, respectively. All CHB patients, from both groups, who did not show VB, exhibited b2m elevation in the third month of treatment. In comparison to patients from group A whose b2m levels were increased at 3 months, patients whose b2m levels were decreased, had 4.6 times higher risk of experiencing VB (RR = 4.6, 95% CI, 1.22–17.36). When the pre-treatment variables were evaluated, decreased b2m status was still associated with increased risk of VB (RR = 12.2; 95% CI, 1.28–116.8).
Conclusions: In CHB patients under long-term LAM monotherapy or initial combination treatment, serum b2m levels at 3 months of treatment, compared with baseline ones, are a good predictor of risk for VB. The initial combination treatment seems to delay or prevent the emergence of YMDD variants, irrespective of baseline parameters.

P1073 Psychiatric adverse effects of interferon alpha 2a treatment in chronic hepatitis patients

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Objectives: To investigate the adverse effects of interferon alpha 2a treatment in chronic hepatitis patients.
Method: Patients diagnosed to have either chronic hepatitis B or chronic hepatitis C were admitted to this study. Patients with chronic hepatitis B received interferon alpha 2a 9MU while chronic hepatitis C patients received interferon alpha 2a 3MU three

times a week. We planned to apply Hamilton Depression Rating Scale (HDRS), Hamilton Anxiety Rating Scale (HAM-A), Montgomery–Asberg Depression Rating Scale (MADRS) and Beck Depression Inventory (BDI) to the patients before the onset of treatment and at the second, fourth and sixth months of treatment period. Depression was diagnosed with scores 7 or higher in HDRS, 14 or higher in MADRS and 13 or higher in BDI. Wilcoxon test was used in statistical analyses.
Results: Here we present the results of first 2 months of the study period. Totally 31 patients 67.7% ($n = 21$) men and 32.2% women ($n = 10$) were involved in the study. Mean age was 40.80 ($\pm$12.92). Statistically significant increase in HDRS, HAM-A, MADRS and BDI scores was noted at the second month of therapy ($P < 0.05$). Depression was diagnosed at the beginning of the treatment with HDRS scores in 22.7% ($n = 7$), with MADRS in 12% ($n = 4$), with BDI in 29% ($n = 9$), whereas this was increased at the second month of therapy to 41.9% ($n = 13$), 19% ($n = 6$), 48% ($n = 15$), respectively.
Conclusions: Psychiatric findings are common in chronic hepatitis patients. Interferon treatment also contributes to the psychiatric findings, which is why psychiatric evaluation of patients undergoing treatment is fundamental.

P1074 The prevalence of hepatitis B virus precore mutant in patients with chronic hepatitis B virus infection

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Objectives: The aim of this study was to determine the prevalence of hepatitis B virus (HBV) precore mutant in chronic hepatitis B patients. HBV with a mutation G to A point mutation at nucleotide 1896 in the precore region is reported to be associated with chronic progressive hepatitis B.
Methods: The presence of precore mutant was examined by amplification refractory mutation detection system (ARMS) in 63 chronic hepatitis B patients and 45 asymptomatic HBV carriers. The precore stop codon mutant and wild type HBV were amplified using two different upstream primers which were different only at the 3′ end at nucleotide 1896.
Results: HBV precore mutant was found in seven of 35 (20%) HBe antigen (HBeAg) positive patients with chronic hepatitis and 22 of 28 (78.5%) antiHBe positive patients. Among 45 asymptomatic HBV carriers, 24 were HBeAg positive and 21 were antiHBe positive. Precore mutant was detected in two of 24 (8.3%) HBeAg positive carriers and nine of 21 (42.8%) anti-HBe positive carriers.
Conclusion: This study suggest that the patients with chronic HBV infection in our area were frequently infected with precore mutant and the overall prevalence of precore HBV mutant with 46% (29/63) in chronic hepatitis B patients was higher than the prevalence of 24.4% (11/45) in asymptomatic carriers.

P1075 Antigenic property of different sequence variants of the hepatitis B surface antigen wild types and vaccine escape mutants

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Objectives: The purpose of this study was to determinate and evaluate of sequence heterogeneity on antigenic properties of hepatitis B surface antigen (HBsAg).
Methods: A number of recombinant HBsAg wild subtypes adw2, adw4, ayw1, ayw2, adr as well as 'vaccine escape mutants' adw2 T126S, Q129R, Q129L, T143K, Q145R and ayw1 Q145A were synthesised, purified and tested by enzyme immunoassay with a panel of 43 commercial available monoclonal antibodies specific for different determinants of HBsAg.
Results: Recombinant proteins were tested at the same concentration 1 ng/μL. All recombinant HBsAg proteins were immunoreactive and demonstrated very different level of reactivity. Among wild subtypes mostly immunoreactive with this panel was recombinant HBsAg subtype adw2. This protein has been detected by 90.6% of used MABs. Average S/Co was 43.7. Recombinant HBsAg subtype adw4 was least immunoreactive (55.8% MABs and average S/Co 3.7). Point mutations affected very different on HBsAg antigenic properties. The immunoreactivity of 'vaccine escape mutants' strains with MABs panel varied from 88.4 to 34.8%.
Conclusion: These data suggest that HBsAg sequence heterogeneity has a significant effect on the antigenic properties of this antigen. Diagnostic test development requires careful selection of MABs as diagnostic reagents. Recombinant HBsAg point mutants, especially 'vaccine escape mutants', may be used efficiently for evaluation of sensitivity commercial and 'in house' EIA for HBsAg detection.

P1076 Performance of hepatitis B surface antigen (HBsAg) assays for the detection of recombinant and native HBsAg mutants and screening for HBsAg mutants in isolated anti-HBc positive sera

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Objectives: The genetic variability of hepatitis B virus (HBV) represents a challenge for the sensitivity of immunodiagnosis, especially for the detection of surface antigen (HBsAg). Mutant viruses may escape detection by commercial HBsAg kits. The aim of the present study was to evaluate the sensitivity of commercial immunoassays for the detection of HBsAg mutants and to screen for mutants in samples with isolated reactivity to core antigen (anti-HBc).
Methods: Four commercially available assays VIDAS HBsAg Ultra (bioMérieux); Liaison HbsAg (Diasorin), Immulite HbsAg (DPC) and Elecsys HbsAg (Roche Diagnostics), HbsAg (V2) (Axsym, Abbott) were tested with different types of HBsAg mutants: (i) HBsAg subtype ayw3 mutants that were obtained by site directed mutagenesis, each mutant contained single amino acid (aa) substitutions or insertions in the 'a' determinant from aa 122 to 147 ($n = 12$). (ii) COS cell supernatants from cloned mutants with s single or multiple aa substitutions ($n = 6$). (iii) Native mutants from patients with chronic hepatitis B or liver transplant recipients with antiviral or hepatitis B immune globulin therapy ($n = 5$). A total of 190 isolated anti-HBc positive samples were screened with the five assays in order to detect any HBsAg mutant or low-level surface antigen carrier.
Results: The sensitivity of the HBsAg assays for mutant virus detection was highly variable, ranging from 17 (73.9%) (Elecsys HBsAg) to 22 (95.7%) mutants detected (VIDAS HBsAg Ultra). VIDAS HBsAg Ultra failed to detect one of five native mutants. Two alternative assays detected all the five native mutants, but they showed a lower performance for recombinant mutants than VIDAS HBsAg Ultra. No positive result was obtained among the 190 isolated anti-HBc positive sera. Eight (4.2%) samples tested positive with real-time PCR showed low viral loads ranging from 20 to 400 IU/mL.
Conclusion: Recombinant mutant Ag could be a useful tool as an indicator of the ability of the kits to detect mutated Ag. Nevertheless, no correlation could be established between the performances observed with recombinant toward native mutant Ag. Since only a limited number of mutants were tested, the data obtained in the present study do not exactly reflect the performance of the assays in large field trials. There was no evidence for the presence of S gene mutants in isolated anti-HBc positive samples.

P1077 Hepatitis B virus genotypes in the Czech Republic

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Objectives: The knowledge of genetic heterogeneity of hepatitis B virus (HBV) is important for epidemiology of this virus also. Sequence analysis of S gene PCR products enables to study phylogeny and to determine genotypes of HBV. The aim of study was to find out the heterogeneity of S gene of HBV from hepatitis B patients in the Czech Republic and to determine the prevalence of HBV genotypes, basic epidemiologic features and geographic distribution.
Methods: HBV S gene DNA amplified by PCR from sera of 176 hepatitis B patients was sequenced. Consensus nucleotide sequences were obtained using CLUSTAL W (European Bioinformatics Institute, GB) followed by manual editing using Bioedit 5.09. Phylogenetic trees were constructed with MEGA 2.1 using UPGMA Kimura two-parameter model.
Results: HBV DNA sequences from 176 patients and 39 GeneBank HBV DNA sequences of known genotype (A–H) were used to construct a phylogenetic tree. Genotype A was determined in 118 (67.1%), genotype D in 50 (28.4%), genotype B in 6 (3.4%), genotype C in 2 (1.1%) of 176 patient HBV. There was no significant difference in prevalence of genotypes A and D in males and females, Prevalence ratio of genotype A/D is different in the age group 10–29 years (56.7%/43.3%), age group 30–59 years (80.4%/19.6%) and age group 50+ years (75.4%/24.6%). There are no geographical differences in the prevalence of genotypes between Bohemian, Moravian and Prague regions. In the cluster of genotype A in the phylogenetic tree 31% of sequences were identical. Out of six patients with genotype B four were Vietnamese, two were native Czechs, one patient with genotype C was Chinese.
Conclusions: There are two predominant HBV genotypes A and D in the Czech Republic without significant differences in their geographic distribution. Genotypes B and C were found with low prevalence. Genotypes B and C were found within Asian immigrants, and rarely in patients native to the Czech Republic also.
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P1078 High prevalence of HFE gene mutations in patients with hepatitis B and other liver diseases in the Czech Republic

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Aim of study: To establish the prevalence of HFE gene mutations in population of Czech Republic and among patients with common liver diseases.
Patients and methods: Altogether, 339 patients including 35 patients with haemochromatosis, 76 with alcoholic liver cirrhosis, 45 with chronic hepatitis C, 13 with chronic hepatitis B, 43 with steatohepatitis, 41 with cryptogenic liver disease, 42 with Dupuytren's contracture, 44 with diabetes and 257 randomly selected 13-year-old Guthrie cards were examined. The HFE gene mutations (C282Y and H63D) were screened for by restriction enzyme analysis performed on PCR amplified products.
Results: In the control group we found 7.8% of C282Y heterozygotes and 26.8% of H63D heterozygotes. In patients with haemochromatosis we found 57% of C282Y ($P < 0.0001$) and 14% of H63D homozygotes ($P < 0.01$). We detected an increased prevalence of heterozygotes for H63D mutation in comparison with the control group in patients with hepatitis B (56.5%, $P < 0.001$), in Dupuytren's contracture patients (35.7%) and in diabetics (27.3%). An increased percentage of both C282Y and H63D homozygotes was found in patients with aetiologically unclear liver disease (9.8%, $P < 0.001$; 4.9%, $P < 0.05$) and of H63D homozygotes in patients with alcoholic cirrhosis (7.9%, $P < 0.001$) and steatohepatitis (7%, $P < 0.05$).
Conclusions: HFE gene mutations are frequent in patients with common liver diseases and hepatitis B. Therefore, we recommend that patients with these diseases be genetically examined for haemochromatosis.
Acknowledgement: Supported by the research goal 002 of the Third Faculty of Medicine, Charles University.

P1079 Performance of AxSYM HBsAg in a Danish patient population

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Objectives: Patients with very varying pre-test probability for hepatitis B virus (HBV) infection are often examined by the same assay for detection of hepatitis B surface antigen (HBsAg). An important subset is patients recently exposed to needle accidents, who receive the first HBV vaccination before blood sampling for the test. We examined if confirmation of the primary test result always is needed, in which situations other tests than detection for HBsAg is preferred, and whether the recommended cut-off level on 2.0 S/N is optimal in our Danish patient population.
Methods: 3229 blood samples from a period of 15 month were analysed for HBsAg first by AxSYM HBsAg Abbott immunoassay principle, second were samples with S/N value on at least 1.85 in the AxSYM HBsAg assay reanalysed by the VIDAS HBsAg immunoassay, and lastly confirmed by the VIDAS HgsAg confirmatory assay. This revealed 3084 negative tests and 145 positive tests. The final results of these tests were characterised by plotting into a Probit-curve. Further, all variables indicating seroconversion were registered for 20 patients with positive test results just above the applied cut-off level on 1.85 S/N and 30 patients with results just below this limit.
Results: The Probit-curve showed a sigmoid curve with 50 samples surrounding the cut-off point, situated in the horizontal middle part of the curve. Nine of these patients had positive results in both AxSYM and VIDAS even though none were infected. Instead, seven of them were persons exposed to needle accidents, and therefore vaccinated against HBV. The present test had in our patient population a positive predictive value of 93.8% and a negative predictive value of 99.9%.
Conclusions: By excluding patients, who had just received HBV vaccination, from HBsAg tests, and by adjusting the cut-off point of AxSYM immunoassay from 1.85 S/N to the recommended 2.00 S/N, we had a gain in the positive predictive value of 99.3% with the same high negative predictive value of 99.9%. In our patient population, we found no benefit by confirming samples with AxSYM values higher than 40 S/N. Due to this study, our laboratory is now able to perform HBsAg analyses with a much higher positive predictive value and to a lower cost.

P1080 Cytomegalovirus acute viral hepatitis – results of a 6-year study (1998–2003) after introducing CDC case definitions and hepatitis B vaccination

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Objectives: To establish the diagnostic hints and clinical picture for cytomegalovirus acute viral hepatitis in the Cluj-Napoca Teaching Hospital of Infectious Diseases after introducing the hepatitis B vaccination in the National Vaccination Programme (1995) and the confirmatory serological testing for acute viral hepatitis A, B, C (1998).

Methods: We retrospectively studied all acute viral hepatitis (2842 cases) admitted in the Cluj-Napoca Hospital of Infectious Diseases between 1998 and 2003. We designed a database using the medical records comprising of: demographic data, premorbid conditions, questionnaire about possible nosocomial and other exposures, bilirubin, ALT, other biochemical tests, the main serological markers for AVH (IgM anti-HAV, IgM anti-HBc, third generation HCV EIA). In cases of unknown type serology for Epstein–Barr virus, cytomegalovirus, *Toxoplasma gondii* were performed as other examinations if considered (2001–2003). EPI6 software was used for statistical analysis.
Results: The incidence rates of acute viral hepatitis type B were significantly decreasing representing 408 cases, 14% of all cases, while type C and non-A–C acute viral hepatitis demonstrated an increasing trend with 370 cases, 13% of all cases. We found 19 cases of cytomegalovirus acute hepatitis based on exclusion criteria for presumable aetiology, on positive serology (IgM positivity, seroconversion). The majority of cases were found in immunocompetent teenagers and adults (16 cases), three cases occurred in renal transplant recipients coincident with positive serological markers for types B and C hepatitis probably representing viral reactivation. In all teenagers the presence of radiographic interstitial pneumonitis (with no or mild clinical signs), enlargement of lymph nodes and spleen were found. The onset duration was quite long (range between 14 and 30 days) and the evolution in all cases was moderate but prolonged (moderate increase in ALT levels). In striking contrast with the literature prolonged jaundice and hiperbilirubinaemia were described in immunocompetent cases (even in teenagers). Immediate prognosis was good but cases occurring in adults showed a clinical and biochemical unsatisfactory evolution.
Conclusion: The WHO eradication project of hepatitis B demonstrates very good results with almost no cases in children, under a good notification of cases. Hepatitis C and other aetiologies are increasingly found affecting immunocompetent subjects.

P1081 Results of vaccination against VHB and VHA in HIV-positive patients

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Objectives: In connection with quality of life improvement and long-term survival of HIV-positive patients in HAART era there has been relative morbidity and mortality increase on liver diseases within this particular population. Therefore we started a vaccination programme against VHB and VHA in selected patients in AIDS Center of the University Hospital Na Bulovce Prague.
Methods: Patients with CD4 lymphocytes over 200/μl and with negative markers of current or past infection (anti-HAV IgG-, anti-HBc IgG- and HBsAg-) have been included in the vaccination programme. The vaccines of the Engerix, Havrix and Twinrix types were used based on regular vaccination schemes. Seroconversion of anti-HAV IgG or anti-HBs with continuing antibody titres over 10 IU/L in anti-HAV IgG and over 10 IU/L in anti-HBs in the period of 6 months after the completion of vaccination has been considered as a positive response.
Results: 25 patients have been vaccinated against VHA. We recorded positive response in 21 of them (84.0%). Twenty-six patients have been vaccinated against VHB. Positive response was recorded in 18 patients (69.2%).
Conclusions: HIV-positive patients in our cohort responded significantly better to vaccination against VHA than to vaccination against VHB. Although serologic response to vaccination is worse than in general population, we support generally accepted recommendation of vaccination in HIV-positive patients against VHA and VHB.

P1082 Evaluation of the Artus HBV LightCycler kit for quantitative detection of hepatitis B virus in serum samples

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Objectives: To evaluate the Artus HBV LightCycler kit for the quantitative detection of hepatitis B virus (HBV) in serum samples in comparison with the Roche COBAS system.
Methods: The Artus HBV LightCycler kit is designed to amplify a 290-bp sequence of the HBV genome. An internal control, included in the kit, was added to serum samples ($n = 50$) to monitor extraction as well as amplification inhibition. Quantitative data were compared with that generated by the Roche COBAS system.
Results: Thirty-three (67%) samples were positive by both methods (one sample was insufficient for COBAS analysis). Two samples that were positive by COBAS but negative by Artus were both low copy number samples (2×10^2 and $<2 \times 10^2$ copies/mL). One additional low copy number sample (3×10^2 copies/mL by COBAS assay) could not be quantified with the Artus kit. Amplification of the internal control was demonstrated for all of the negative samples. One-third of the positive samples (12/36) exceeded the dynamic range of the COBAS assay. Removing these samples from analysis, there was <1 log variation between the two assays for 16/24 (67%) of the comparable samples. Five samples were retested to assess intra-assay and batch-to-batch reproducibility. Variation for samples containing 10^3 –10^7 copies/mL was <0.5 log, whilst the low copy number sample (10^2 copies/mL) displayed <1 log variation over the three runs.
Conclusions: Overall, results obtained with the Artus kit on the LightCycler were in agreement with the COBAS data. Although the Artus kit appeared to be slightly less sensitive, a greater dynamic range was demonstrated.

P1083 Correlation of circulating endothelin-1 and nitrates/nitrites with the liver histopathological staging and grading in patients with chronic viral hepatitis B

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Objectives: To estimate the endotheline-1 (ET-1) and nitric oxide (NO) metabolism products (nitrite and nitrate) and correlate them with the liver histopathological grading and staging in patients with chronic hepatitis B.
Methods: From September 2000 to September 2002, 32 patients (21 men and 11 women) with chronic hepatitis B and 30 healthy volunteer control persons (14 men and 16 women) included in this study. Mean age was 33.3 (18–59) in the study group and 31.6 (18–46) in the control group. Clinical diagnostic criteria for chronic hepatitis B were positive test result of HBsAg for minimum 6 months and concomitant elevated serum alanine transaminase (ALT) levels. Liver biopsies have been performed for all patients. Histopathological activity were scored as minimal (1–4), mild (5–8), moderate (9–12), severe (13–18) and 0–6 according the activity and fibrotic index, respectively, using classification of chronic viral hepatitis described by Knodell *et al*. ET-1 serum concentration was determined with a commercially available ELISA assay kit according to the instructions of manufacturer. Nitrite was measured by using Griess reaction. Nitrate concentration of the serum was calculated by subtracting nitrite measured from total nitrite.
Results: There were no significant differences in age and sex distributions between chronic hepatitis B patients and control groups ($P > 0.05$). Serum ET-1 levels were higher in patients with chronic hepatitis B (9.43 ± 3.45 pg/mL) than the healthy voluntary people (2.9 ± 0.86 pg/mL) ($P < 0.0001$). ET-1 levels correlated well with the severity of the viral hepatitis using the

Knodell's liver histological activity index ($r = 0.440$, $P = 0.046$). There was a significant increase of serum nitrite and nitrate levels in patients with chronic hepatitis B compared with the control group ($P < 0.001$ and $P < 0.01$). In addition there was a positive correlation between serum nitrite/ET-1 ($r = 0.719$, $P = 0.001$) and nitrate/ET-1 ($r = 0.651$, $P = 0.005$) levels in chronic hepatitis B patients but the same correlation was not found in the control group.
Conclusions: It was found that there was a positive correlation between serum nitrite, nitrate and ET-1 levels with liver histopathological grading and staging in patients with chronic hepatitis B. Serum ET-1 levels may be useful clinical indicator for use in the follow-up of patients with chronic hepatitis B.

P1084 Hepatitis D super infection that developed due to endoscopy

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Objectives: Hepatitis D (HDV) is a defective virus. It uses hepatitis B surface antigen (HBs) for being an infectious agent. In chronic HBV cases if super infection develops it leads to chronic HDV in 90% of the cases. In a follow-up study of three sustained responded chronic HBV patients to therapy with interferon plus lamivudin were analysed and HDV superinfection was observed after endoscopic instrumentations.
Methods: Anti HDV positivity and elevations in liver enzymes were observed in three patients aged between 38 and 53. They had been analysed with endoscopy. One case had been analysed with maxillary sinus endoscopy and two cases with gastroendoscopy.
Results: After 8–18 weeks anti-HDV IgG–IgM positivity was observed by EIA (Sorin Italy). There was no way of transmission of HDV. Three cases were diagnosed as chronic HDV through clinical, virological (anti-HBs IgM and HBV-DNA negative) and histological (liver biopsy) techniques. Ten million units interferon has been used for 1 year three times a week. At the and of treatment in both these cases anti-HDV markers were negative and liver enzymes of patients were normal and only one patient had positive anti-HBs:25 IU/mL. Other two cases have been followed for HBs, anti-HBe, anti-HBc IgG which were positive, but HBV-DNA negative and live normal level.
Conclusions: Endoscopic trials in chronic HBV patients can be a transmission way of HDV superinfection.

P1085 The epidemiology and phylogeny of TT virus in the Czech Republic

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Objective: TT virus (TTV) is prevalent worldwide with the highest rate in African and South American countries. The prevalence of this virus is higher in people with a risk of parenteral acquisition of infection but other routes of transmission are being investigated. This virus is suspected to be an aetiological factor of non-A-E hepatitis but so far most studies have failed to support this hypothesis. In our study we investigated the epidemiology of TTV in the Czech Republic.
Methods: The following groups of subjects were enrolled: a control group consisting of 196 blood donors, 20 patients with haemophilia, 49 drug users, 100 prostitutes, 50 prisoners, 208 healthy children aged 1–14 years, 54 cord blood samples, 52 patients with non-A-E hepatitis, 74 patients with hepatitis C and 51 blood donors with increased ALT level. Two sets of primers, specific for the open reading frame 1 (ORF1) and non-coding region (NCR) region, were used.
Results and conclusions: In the control group the prevalence was 7.1 and 52.6%, respectively. Patients with haemophilia, intravenous drug users (IVDUs), prisoners and patients with hepatitis C had a significantly higher prevalence of TTV DNA regardless of the set of primers used. Additionally, using the primer set for NCR, patients with non-A-E hepatitis had also a significantly higher prevalence of TTV. We did not detect any TTV DNA in the cord blood specimens but we observed an increase of TTV DNA prevalence with age both in children and adults. Phylogenetic analysis of 70 isolates revealed that the most prevalent genotypes in the Czech population are G2 and G1, followed by G8 and G3. Our study subjects infected with HBV and/or HCV were significantly more often TTV DNA positive, suggesting a common route of transmission of these viruses.

P1086 Absence of occult HCV and HBV infections in haemodialysis patients

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Objectives: Haemodialysis patients are a risk group for HBV and HCV infections. Occult HBV infection is defined by detection of HBV-DNA in absence of HbsAg. Occult HCV infection is defined by presence of HCV-RNA in absence of anti-HCV. The aim of this study is to determine the prevalence of occult HBV and HCV infections and the diagnostic utility of anti-HCV and HBsAg as markers of HCV and HBV infections in haemodialysis patients since it has important implications in the dissemination of these diseases.
Methods: We have studied prospectively 61 patients (33 males, 28 females, mean time in haemodialysis 41 months, range 1–256, mean age 65 years, range 28–83). Blood samples were drawn before dialysis treatment. HBsAg, anti-HBc, anti-HBsAg and anti-HCV were studied by an automated EIA (Asxym, Abbott Diagnostics). Detection of HBV-DNA and HCV-RNA was performed by an automated PCR system (Ampliprep and Amplicor, Roche Diagnostics). The limit of detection is 200 c/mL and 50 IU/mL, respectively.
Results: HBsAg was detected in none of the patients (0%). Anti-HBc and anti-HBs were detected in seven patients (11.5%) and anti-HBc alone in two patients (3.3%). Anti-HBsAg was present in 35 patients (57.4%). HBV-DNA testing was performed on all the samples and none resulted positive. Anti-HCV was detected in seven patients (11%). HCV-RNA testing was performed on all the samples (anti-HCV positive and anti-HCV negative) with six being positive. These six positive HCV-RNA samples were also anti-HCV positive. None of the anti-HCV negative patients has HCV-RNA in serum.
Conclusion: Prevalences of HBV-DNA in absence of HBsAg as variable as 19, 36 and even 58% have been reported in haemodialysis patients. The authors have studied the presence of HBV-DNA by in-house assays. Prevalence may vary depending on the assays used to detect HBV-DNA. Regarding HCV infection, some investigators have found prevalence rates of 9% of HCV-RNA positivity without anti-HCV in the serum of haemodialysis patients. Other authors have not confirmed these data. In our study, the prevalence of occult HBV and HCV infection is null. HBsAg and anti-HCV are very useful markers for identification of potentially infectious cases of HBV and HCV hepatitis in the haemodialysis setting.

Streptococci: resistance and epidemiology

P1087 *Streptococcus pyogenes* resistance to erythromycin and selection pressure of macrolides

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Objectives: The aim of the study was the evaluation of the *Streptococcus pyogenes* resistance to erythromycin in Olomouc region (population 300 000), Czech Republic, in association with selection pressure of macrolides.
Methods: During the period of 1997–2002, *S. pyogenes* strains were isolated from oropharyngeal smears of community patients suffering from bacterial tonsillitis. The susceptibility to antibiotics was assessed by disk diffusion method in accordance with the NCCLS guideline. Utilisation of macrolides was obtained from the data of regional General Health Insurance Company and expressed in defined daily doses per 1000 patients per day.
Results: There was a significant increase ($P = 0.05$) in the occurrence of erythromycin-resistant *S. pyogenes* strains from 2% in 1997 up to 33% in 2001, whilst in 2002 they dropped remarkably ($P = 0.05$) to 17%. Utilisation of macrolides increased by 13% in the period of 1997–2001 (from 2.69 to 3.05 DDD/1000/day), their utilisation represented 11.40% of total antibiotic prescription in 1997 and 15.48% in 2001. In 2002, macrolides consumption decreased to 1.89 DDD/1000/day, which represents 10.41% of total antibiotic utilisation.
Conclusion: The study documented a probable association between macrolide utilisation and the frequency of erythromycin-resistant *S. pyogenes* strains. Absolute susceptibility of *S. pyogenes*, which is the most important bacterial pathogen in community-acquired bacterial tonsillitis, to penicillin contrasts with macrolide resistance. It seems reasonable that penicillin should be an antibiotic of first choice in bacterial tonsillitis, macrolides should be reserved for patients allergic to penicillins only.
Acknowledgement: Supported by the MSM 151100002.

P1088 Susceptibility of *Streptococcus pyogenes* to telithromycin and erythromycin: results from PROTEKT (years 1–3)

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Objectives: The increasing prevalence of resistance among *S. pyogenes* to macrolide antibacterials is a cause for concern, particularly as macrolides are considered the main treatment alternative for penicillin-intolerant patients with tonsillitis/pharyngitis caused by *S. pyogenes*. Data from the PROTEKT study – a global, longitudinal study to assess the antimicrobial susceptibility of common bacterial pathogens associated with community-acquired respiratory tract infections (RTIs) – have been analysed to assess the prevalence of macrolide resistance among clinical isolates of *S. pyogenes* collected over 3 years and to assess the activity of the ketolide antibacterial telithromycin (TEL) against such isolates.
Methods: The MICs of clinical isolates of *S. pyogenes* collected worldwide as part of the PROTEKT programme over three consecutive respiratory seasons (1999–2002) were determined centrally by NCCLS broth microdilution methods. Isolates were tested for the presence of macrolide resistance genes erm(B), mef(A) and erm(A) subclass erm(TR) using PCR.
Results: A total of 5034 isolates of *S. pyogenes* were collected between 1999 and 2002 from 34 countries. Over the 3 years, the prevalence of resistance to erythromycin (MIC $\geq$1 mg/L) was 12.1% (10.4, 9.8 and 14.9% in years 1, 2 and 3, respectively). Rates of resistance varied considerably between countries, ranging from 0% (Ecuador and The Netherlands) to 90.9% (40/44) in China [data from years 1 and 3 (one centre only)]. Of the 622 isolates analysed by PCR, 48.9% (304) tested positive for the mef(A) gene, 23.8% (148) were positive for erm(B), 26.2% (163) were positive for erm(A) subclass erm(TR) and 1.1% (7) were negative for the mechanisms tested. TEL retained potent *in vitro* activity against *S. pyogenes*, with 97.5% (4907/5034) of isolates susceptible at concentrations $\leq$0.5 mg/L. Over the 3 years, no major changes in susceptibility to TEL were observed: TEL mode MIC against *S. pyogenes* was 0.015 mg/L for all 3 years, with an MIC90 of 0.03 mg/L in year 1, 0.015 mg/L in year 2 and 0.25 mg/L in year 3.
Conclusion: Approximately 12% of *S. pyogenes* isolates collected worldwide were resistant to macrolides, with mef(A)-mediated resistance the most prevalent mechanism. In contrast, TEL displayed high activity against *S. pyogenes* (>97% of isolates susceptible), including strains resistant to erythromycin.

P1089 Antibiotic susceptibilities of invasive group A streptococci in England, Wales and Northern Ireland: a comparison of results between hospital laboratories and the national reference laboratories

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Objectives: To compare antimicrobial susceptibilities of invasive GAS isolates reported from hospital laboratories with data generated from the Streptococcus and Diphtheria Reference Unit (SDRU) and the Antibiotic Resistance Monitoring and Reference Laboratory (ARMRL). The distribution of antibiotic resistance will also be determined against denominator and typing data (M or emm types).
Methods: Laboratories in England, Wales and Northern Ireland report cases of invasive GAS disease weekly to the Communicable Disease Surveillance Centre. This includes demographic, clinical and microbiological data. Isolates are also submitted to the national reference laboratories and subjected to M typing and antimicrobial testing. Datasets from both sources during the period January to July 2003 were reconciled and analysed. Data used in this analysis included age (in 10 year bands), sex, laboratory, region, site of isolation and GAS type.
Results: A total of 812 invasive GAS isolates were reported by hospital laboratories and received by SDRU between January and July 2003; 723 of these had antimicrobial results. A total of 98.3% (711/723) had been tested by both hospital laboratories and ARMRL; the susceptibilities correlated exactly for all four antibiotics. No penicillin resistance was reported, but 0.7% (5/723) were resistant to clindamycin, 4.0% (29/723) to erythromycin and 13.6% (98/723) to tetracycline. Furthermore, six erythromycin and tetracycline resistant strains were reported, three erythromycin and clindamycin resistant, and two were erythromycin, tetracycline and clindamycin resistant strains. Tetracycline resistance was higher in the younger age groups, peaking at 22.0% (29/132) in 30–39 years olds ($P = 0.09$). Tetracycline resistance was observed in 16.8% (59/351) males compared with 9.3% (29/311) females ($P = 0.018$). GAS M types emm43 (10/11), M77 (4/5), M83 (28/31) and emm91 (3/3) appeared to be associated with tetracycline resistance ($P < 0.000$).
Conclusions: An improvement was noted in the number of invasive GAS cases reported from laboratories, which included antibiotic susceptibility data; from 81% in 2002 to 87.6% during January to July 2003. There was also excellent correlation between these data and the ARMRL results. Erythromycin and tetracycline resistance rates were similar to previous years.

P1090 Macrolide resistance rates and resistance phenotypes of invasive *Streptococcus pyogenes* isolates in Greece

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Introduction: The importance of invasive infections caused by *Streptococcus pyogenes* is due to their severe clinical features and significant increase in occurrence in recent years. The goal of this presentation was the phenotypic and genotypic study of invasive disease isolates, collected in Greek Hospitals, during the period January to October 2003.
Materials and methods: A total of 30 isolates obtained from six Greek Hospitals were studied. Susceptibility to penicillin, erythromycin, clindamycin, vancomycin, cefotaxime, cloramphenicol and tetracycline was tested by a disk diffusion method and interpreted according to NCCLS criteria. Macrolide-lincosamide-streptogramin (MLS) resistance phenotypes were determined by the double-diffusion test using adjacent erythromycin and clindamycin disks. Detection of resistance genes mefA, ermTR and ermB was carried out using the polymerase chain reaction (PCR).
Results: Of the 30 isolates from 14 male and 16 female patients, 13 had been isolated from blood, nine from deep abscesses, three from synovial fluid, two from cutaneous wounds, two from pleural fluid and one from cerebrospinal fluid. The patients' mean age was 19.6 years (range 1–76 years). No resistance to penicillin, vancomycin, cefotaxime and chloramphenicol was detected. Five isolates (17%) were resistant to erythromycin (EryR), whereas eight (27%) were intermediately or fully resistant to tetracycline. Clindamycin resistance was detected only in two EryR isolates, both displaying the IR-MLSB phenotype. The remaining three EryR isolates belonged to the M phenotype. The mefA and ermTR genes were detected in all isolates with M and IR-MLSB phenotypes, respectively.
Conclusion: Invasive *S. pyogenes* strains in Greece remain susceptible to penicillin. Erythromycin resistance was detected among 17% of the isolates, lower than the average resistance rate (27%) recorded previously on 2002.
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P1091 Large differences in resistance to erythromycin in *Streptococcus pyogenes* isolates of pharyngitis among regions in Spain

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Objectives: Resistance to erythromycin in *S. pyogenes* must be monitored in order to ensure the adequacy of the empiric use of macrolides in the treatment of acute bacterial pharyngitis. Variable rates of resistance have been reported around the world and in Europe they are higher in the West Mediterranean region. In our last surveillance (1998–1999) we described a decrease in resistance up to 20%. We wanted to check whether this decrease is confirmed in 2001–2002 similarly to what happens with resistance to penicillin in pneumococci.
Methods: A prospective, multicentre (25 hospitals) antimicrobial survey was carried out between November 2001 and October 2002. A total of 3374 consecutive *S. pyogenes* isolates from adult and paediatric patients with acute bacterial pharyngitis were collected and sent to a central laboratory for further processing. Susceptibility testing was then performed by a semiautomated microdilution method following NCCLS M100-S12 guidelines and breakpoints against antibiotics commonly used.
Results: 70% of the strains were paediatric and 30% from adults. All strains had a penicillin MIC of less than or equal to 0.015 mg/L and therefore considered as susceptible to betalactams. However, the activity displayed by either erythromycin, clarithromycin and azithromycin, which were equivalent to each other, was not optimal. We documented large variations in the prevalence of resistance among regions (Autonomous Communities), with a mean erythromycin resistance rate (and therefore of clarithromycin and azithromycin) of 33.2% (95%CI: 24.7–41.8%). Only Northern regions (Cantabria: 18.9%, Basque Country: 20.8%, Galicia: 25.4%) and Madrid (25%) had a rate below the national mean. Aragon, Andalucia and Cataluña had resistance rates between 30 and 35%, Castilla-León had 40%, Murcia 46.3% and Castilla-La-Mancha 63.8%.
Conclusions: (1) We have not confirmed the decrease in erythromycin (clarithromycin and azithromycin) resistance that seemed to have happened during the late 1990s. (2) On the contrary, what we have seen is an alarming mean increase in resistance (33.2%) with very concerning hot spots in some regions (above 40%). Only a few northern regions remain around 20%. (3) In view of these results, erythromycin, clarithromycin and azithromycin should not be prescribed empirically but given only once a susceptibility testing has been done.

P1092 Erythromycin-resistant *Streptococcus pyogenes*

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Streptococcus pyogenes is the most prevalent cause of tonsylopharyngitis in children. The drug of choice for infections caused by this organism is penicillin. The problem with treating such infections arises when erythromycin-resistant strains occur.
Objectives: The aim of the study was to determine the incidence of *S. pyogenes* resistant to penicillin. The organism was recovered from the pharynx of children hospitalised or ambulatory treated at the University Children's Hospital in Belgrade.
Methods: *S. pyogenes* was identified on blood agar, using bacitracin disc, and confirmed by latex agglutination test (Slidex Bio Merieux). Disc diffusion test was carried out to estimate the penicillin sensitivity. Erythromycin disc was used as a screening method to detect erythromycin-resistant *S. pyogenes*. MIC for erythromycin was performed by broth dilution method.
Results: In the study period from January 2001 to December 2003 all 1100 isolates of *S. pyogenes* showed usual level of penicillin sensitivity. In 2001 only 0.45% of isolates were erythromycin-resistant. In 2002 erythromycin resistance was 0.63%, while in 2003 it was 1.09%. MIC for erythromycin was from 1 to 128mg/L. Three strains expressed constitutive and one strain expressed inducible resistance to clindamycin.
Conclusion: According to the results we can conclude that, despite sensitivity to penicillin, resistance to macrolides is the emerging phenomenon. The reasonable use of macrolide antibiotics is necessary to maintain the resistance at the low level.

P1093 Phenotypic and genotypic characterisation of macrolide-resistant *Streptococcus pyogenes* strains isolated in the Czech Republic during two years (2001–2002)

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Objectives: To determine prevalence of MSLB resistance mechanisms among erythromycin-resistant *S. pyogenes* strains by phenotypic and genotypic methods.
Methods: A total of 916 clinical isolates of *S. pyogenes* resistant to erythromycin were collected in 39 microbiology laboratories. Erythromycin susceptibility was tested by the disk diffusion method and strains which formed an inhibition zone <21 mm around the erythromycin disk (15 μg) were sent to the National Reference Laboratory for Antibiotics (NRL). The resistance pheno-

types of all isolates were characterised in NRL by the double-disk test with erythromycin (ERY, 15 μg) and clindamycin (CLI, 2 μg). Antibiotic susceptibility by disk diffusion method was also determined to azithromycin (AZI, 15 μg) and spiramycin (SPI, 100 μg). Presences of MLSB resistance genes (ermTR, ermB and mefA) were tested by PCR and T serotypes were determined in random representatives of each phenotype ($n = 252$).
Results: The prevalence rate of the strains resistant to MLSB antibiotics (constitutive resistance) was 63% and 51% in 2001 and 2002, respectively. The prevalence rate of the strains resistant to ERY, AZI and inducible resistant to SPI and to CLI (inducible resistance) was 28 and 23%. The prevalence rate of the strains resistant to ERY, AZI but susceptible to SPI, CLI (M phenotype) was 9 and 26%. The major prevalent T types among the strains analysed were serotype T 28 (58%), T 12 (10%), T 4 (8%) and T B3264 (8%).
Conclusion: M phenotype, constitutive and inducible resistance to MLSB antibiotics was detected and genes ermTR, ermB and mefA were found among the strains analysed. The major mechanism of MLSB resistance was constitutive with twofold higher prevalence than the inducible mechanism. M phenotype which was the less frequent mechanism in the first year increased three times in second year of the study. T serotype 28 was proved the most frequent serotype associated with constitutive and inducible resistance to MLSB antibiotics. The study showed the dynamic change in prevalence of phenotypes, which are in relation with T serotypes.

P1094 Increasing rate of tetracycline resistance in *Streptococcus pyogenes* in Sweden

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Objectives: *Streptococcus pyogenes*, a major human pathogen, is still considered susceptible to betalactams, but for other relevant antibiotics highly variable resistance rates have been reported. Since the EC funded Strep-EURO project started in September 2002, a total number of 301 invasive and 500 non-invasive control strains were collected from different region of Sweden. One main objective was to study antibiotic susceptibility and type distribution of resistant strains.
Methods: The strains used in this study were clinical isolates from different diagnostic laboratories in Sweden. The invasive strains were mainly blood isolates, but also from other sterile sites, whereas control strains were throat or skin isolates. Strains were maintained frozen at −80 °C in calf serum. The *in vitro* susceptibility to antibiotics was tested by disk diffusion on PDM agar following the instruction provided by the Swedish Reference Group for Antibiotics (homepage: www.srga.org). MICs of resistant strains were determined by the E-test (Biodisk AB) following the recommendations of the manufacturer. T typing was performed by slide agglutination according to previously documented methods using sera from Sevapharma, Prague.
Results: Erythromycin resistance was uncommon (0.6%), whereas an overall high rate of tetracycline resistance was found (25–30%). MIC for tetracycline resistant strains varied between 8 and 64 mg/L with a clustering at 24 mg/L. The tetracycline resistant strains belonged to more than 10 different T types, the majority being types 3/3264, 13, 28 and B3264. Among Invasive strains T-types 3/3264, 3/13/3264 and 28 predominated.
Conclusion: Since in Sweden tetracycline is used in the treatment of chlamydial and mycoplasmal rather than streptococcal infections the level of tetracycline resistance among GAS clinical isolates, 25–30%, appeared comparatively high. However, in certain countries much higher rates were recently reported. To account for resistance development, horizontal gene transfer from chlamydia or mycoplasma seems more unlikely than from other streptococci (groups B, C, D, G) especially with regard to increasing veterinary usage of tetracycline.

Acknowledgments: The Strep-EURO project is funded by the European Commission. We are grateful to diagnostic laboratories providing strains and patient data to Strep-EURO.

P1095 Multicentre evaluation of macrolide and ketolide activities and different genotypes of erythromycin resistance in Italian streptococci

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Objectives: To investigate the actual incidence and nature of macrolide and ketolide susceptibilities among *Streptococcus pyogenes* and *Streptococcus pneumoniae* isolated in Italy.
Methods: The activities of erythromycin and telithromycin, as well as of other reference antibiotics, were assayed on 200 *S. pyogenes* and 188 *S. pneumoniae* isolated in different Italian centres throughout 2003. The presence of known resistance genes – both erm and mef – was investigated by PCR on all resistant strains.
Results: 11.5% of the *S. pyogenes* isolates proved resistant to erythromycin. The genotypic analysis revealed the presence of an erm gene in 78.3%, and of a mef gene in 21.7% of the erythromycin-resistant isolates, respectively. About 27.7% of the *S. pneumoniae* isolates proved erythromycin-resistant, whilst 8% were not susceptible (intermediate) to penicillin. The genotypic analysis showed an erm gene in 86.5% and a mef gene in 13.5% of the erythromycin-resistant isolates, respectively. All the *S. pyogenes* and *S. pneumoniae* erythromycin-resistant strains proved also resistant to azithromycin and clarithromycin. A total of 94% of the *S. pyogenes* isolates and 95.2% of the *S. pneumoniae* isolates were susceptible to telithromycin.
Conclusions: Erythromycin resistance could be attributed to the presence of either an erm or a mef gene in both *S. pyogenes* and *S. pneumoniae* isolates, and the erm gene accounted for the vast majority of resistant isolates in both streptococcal species. As opposed to recent reports, erm and mef genes did not coexist in any strain. Telithromycin resistance was present in both species, though still limited to a few individual strains.

P1096 Incidence of fluoroquinolone-resistant beta-haemolytic Streptococci in North America (NA) and Europe (EU): report from the SENTRY Antimicrobial Surveillance Program, 1997–2002

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Background: Recent publications have reported rare cases of beta-haemolytic streptococci (BHS) with resistance (R) to fluoroquinolones (FQ). These pathogens can cause invasive disease and have generally remained susceptible (S) to the FQ class. This multi-centre investigation was initiated to determine the rate of FQ-R and the responsible quinolone resistance-determining region (QRDR) mutations among BHS.
Methods: The SENTRY Program has tested FQ against BHS in NA and EU since 1997. This study used NCCLS broth microdilution and Etest methods to determine S to ciprofloxacin (CIP), gatifloxacin (GAT), levofloxacin (LEV), garenoxacin (GAR), gemifloxacin (GEM) and moxifloxacin (MOX). Nineteen BHS isolates from NA and EU had CIP MIC results >2 mg/L. Vitek and API 20 strep as well as conventional methods and colony morphology were used to confirm identification. Eleven strains were available for molecular analysis using PCR to determine mutations in the QRDR. Primers were designed to amplify the QRDR of gyrase and topoisomerase genes (gyrA, gyrB, parC, parE) from *S. pyogenes* (BSA) and *S. agalactiae* (BSB) against respective genes found in the GenBank database. PCR products were

sequenced on both strands by the dideoxy-chain termination method and analysis performed using laser gene DNA Star software.
Results: The rate of FQ-R BHS was 0.36% (EU) and 0.46% (NA) during the study period with the highest rate in 2002 (1.7%). These isolates included BSA (8), BSB (8) and *S. dysgalactiae* (BSC/G; 3). The MIC90 for the FQs (mg/L) showed highest potency for: GEM (2) > GAR (4) > MOX (8) > GAT (16) > LEV (>4) > CIP (>32). All strains had significant mutations in either parC (position 79 or 83) and/or gyrA (position 81 or 85). Two BSA strains with lower level R to CIP (4 mg/L) had only parC mutations (Ser79 to Phe). All isolates of BHS with high-level R (>32 mg/L) to CIP had gyrA mutations and often parC mutations. Numerous other mutations in the QRDR region were found including gyrB and parE, although their significance remains unknown.
Conclusions: The increasing rate of FQ-R streptococci including *S. pneumoniae*, viridans group streptococci and more recently reported, BHS, is becoming a clinical concern due to the morbidity and mortality caused by these pathogens. Strains of BHS with high-level R to FQ have point mutations common to other streptococci in gyrA and parC.

P1097 *In vitro* activity of telithromycin against erythromycin-resistant and susceptible viridans group streptococci isolated from blood

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Objectives: Emergence of erythromycin resistance in viridans group streptococci (VGS) has been observed in many parts of the world. We studied the activity of telithromycin against VGS isolated from blood and determined the macrolide mechanism of resistance.
Methods: 155 SGV unique and consecutive strains isolated from blood were included in the study. Isolates were identified at the species level by ID 32-Strep system (Biomérieux, Marcy L'Etoile, France) and then classified according to the review of Coykendall. The antibiotic susceptibility to erythromycin (E), clindamycin (C) and telithromycin (T) was performed by the agar dilution method. The erythromycin resistance phenotypes were determined by the double disc diffusion test and detection of mef(A) erm(B) and erm(A) genes was performed by PCR.
Results: The VGS were identified as: *S. mitis* (68), *S. anginosus* (44), *S. sanguis* (25), *S. bovis* (10), *S. salivarius* (7) and *S. mutans* (1). Overall, the E and C resistance percentages were 52.2 and 32.2%, respectively. Among the 81 E-resistant strains, 62% belonged to the cMLSB phenotype and 38% to the M phenotype. The MIC90 (mg/L) for E, C, and T in the different groups of VGS was: *S. mits* (128/128/0.25), *S. anginosus* (256/256/0.25), *S. sanguis* (128/128/0.12), *S. bovis* (256/256/64) and *S. salivarius* (2/0.06/0.12). Among the 50 cMLSB phenotype strains all were erm(B)+ and five were erm(B)+ and mef(A)+. All M phenotype strains harboured the mef(A) gene. None of the erythromycin-resistant isolates exhibited the erm(A) gene. The most E-resistant group was *S. bovis* (90%) and the less resistant group was *S. salivarius* (14%). All VGS groups showed T MICs <4 mg/L independently of the erythromycin resistance genotype. The exception was the *S. bovis* group with five strains showing telithromycin MIC $\geq$4 mg/L.
Conclusions: We have found a high rate of erythromycin resistance among our VGS and the main macrolide resistance mechanism is mediated by erm(B) gene. Telithromycin was active against all VGS groups, but *S. bovis*, with MIC90 $\leq$0.25 mg/L. The *S. bovis* group was the most resistant to erythromycin and the only that showed telithromycin MIC $\geq$4 mg/L.

P1098 Resistance rates of viridans group Streptococci in children attending day-care centres

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Background: Viridans Group Streptococci (VGS) form the major part of the commensal flora of the human upper respiratory tract and antibiotic resistance is of currently concern among this bacterial population in several settings.
Objectives: To determine colonisation and resistance rates to betalactams, macrolides and fluoroquinolones antibiotics of VGS isolated from 600 healthy children 3 months to 3 years old attending to familial and collective day care centres in Nice (France).
Methods: A total of 63 positive cultures were obtained by nasopharyngeal aspiration. Antibiotic susceptibility was determined by disk diffusion method and E-test on Mueller Hinton agar plates supplemented with 5% sheep blood.
Results: Streptococci were identified by species: 28 *S. mitis*, 19 *G. morbillorum*, 7 *S. oralis*, 3 *S. sanguis*, 1 *A. viridans*, 1 *S. bovis*, 2 *S. adjacens* and 2 *G. haemolysans*. The carriage of penicillin resistant and intermediate strains was in order 36.5 and 25%, according to E-test values and NCCLS recommendations; erythromycin resistance rate was 50%, while no strains telithromycin resistant were found. Among the erythromycin resistant isolates the erm phenotype of macrolide resistance predominated (95%). The new generation fluoroquinolones studied (levofloxacin and sparfloxacin) showed good activity against VGS, while a ciprofloxacin resistance rate of 4.7%was observed.
Conclusions: Penicillin resistance of VGS (61.5%) is equivalent to penicillin resistance of *S. pneumoniae* (58.5%) in this children population, while the comparison of erythromycin resistance in the VGS and *S. pneumoniae* isolates showed a higher level of erithromycin resistance in the pneumococcal isolates (74%) than in the VGS isolates (50%). Newer fluoroquinolones showed good activity towards the bacterial isolates tested, apart from a 4.7% ciprofloxacin resistance rate observed, not easily explicable in this children population and which requires further evaluations.

P1099 The eagle effect revisited. Penicillin resistance in group G beta haemolytic streptococcal infection

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Background: Severe invasive beta haemolytic streptococcal cellulitis requires prompt diagnosis and rapid administration of appropriate antibiotic therapy. Because group A streptococcal infection continues to be exquisitely sensitive to betalactam antibiotics, clinical studies recommend the intravenous administration of Penicillin G. Despite known antibiotic sensitivity to penicillin however, there have been some treatment failures reported. Known as the 'Eagle Effect', penicillin appears effective against group A streptococcus if given early in the infective process or where streptococcal titres are low, however shows a marked reduction in efficacy if the infective process is allowed to progress or the inoculum size is high (Inoculum effect).
Case report: Although well documented in group A streptococcal infection, the Eagle Effect has been less well reported with infections due to other streptococcal serogroups. This report examines a case of cellulitis in an otherwise healthy 47-year-old male where the sole bacterial isolate was a beta haemolytic group G streptococcus. Treatment failure with penicillin was observed despite known culture sensitivity and the addition of clindamycin, gave dramatic clinical improvement.
Conclusion: This finding may implicate an Eagle-Like Effect in poorly responding group G beta haemolytic infections. This has not been previously reported and may have important implications for clinical management.

Resistance in pseudomonas and other Gram-negative bacilli

P1100 Suitability of current routinely generated data for surveillance of antimicrobial resistance of *Escherichia coli* and *Pseudomonas aeruginosa* in the UK and Ireland

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Objective: To assess whether clinical laboratories' routine data can be used for surveillance of antimicrobial resistance. Do routine tests detect resistance reliably, and is there selection bias in the isolates tested with particular antibiotics?
Methods: In 2001 and 2002, 29 local laboratories in the UK and Ireland collected 495 isolates of *E. coli* (EC) and 367 *P. aeruginosa* (PA) for the BSAC Bacteraemia Resistance Surveillance Programme and supplied routine data on them, generated by local methods. Isolates were re-tested centrally with the BSAC agar dilution MIC method and breakpoints. Local test with ampicillin was accepted as an alternative to amoxicillin with *E. coli*.
Results: There were no significant differences in percentage susceptibility by reference tests between isolates tested and not tested locally, implying no selection bias in local testing. Local laboratories' detection rates for susceptibility were high (≥86% for EC and ≥94% for PA, for all antibiotics), but detection rates for resistance varied from 29% (PA/TZP) to 89% (EC/CIP) and averaged only 70% for the organism–agent combinations reported here. Of 183 undetected resistances, local laboratories reported 25 as intermediate and 158 as susceptible. Not all MICs in cases of undetected resistance were near their breakpoints.

Species and drug	*n* of isolates tested, not tested locally	% S for isolates tested, not tested locally	Local detection of S	Local detection of R
EC AMX	475,20	41,40	167/194 = 86%	249/281 = 87%
EC AMC	362,133	76,77	255/274 = 93%	41/88 = 47%
EC CAZ	403,92	97,98	385/392 = 98%	5/11 = 45%
EC CIP	480,15	93,100	442/444 = 100%	32/36 = 89%
EC CXM	401,94	90,91	331/360 = 92%	19/41 = 46%
EC GEN	490,5	89,80	429/436 = 98%	19/54 = 35%
EC IPM	246,249	100,100	246/246 = 100%	n < 10
EC TZP	263,232	95,98	247/250 = 99%	6/13 = 46%
PA CAZ	342,25	96,92	310/330 = 94%	8/12 = 67%
PA CIP	361,6	87,83	308/313 = 98%	23/26 = 88%
PA GEN	362,5	56,60	201/203 = 99%	15/23 = 65%
PA IPM	171,196	91,94	151/156 = 97%	10/15 = 67%
PA TZP	250,117	94,96	224/236 = 95%	4/14 = 29%

AMX amoxicillin, AMC amoxicillin–clavulanate, CAZ ceftazidime, CIP ciprofloxacin, CXM cefuroxime, GEN gentamicin, IPM imipenem, TZP piperacillin–tazobactam.

Conclusion: Routine susceptibility data on *E. coli* and *P. aeruginosa* can be useful for surveillance, but cautious interpretation is needed for some antimicrobials. Further efforts to increase reliability and standardisation are warranted.

P1101 Diversity among 2481 *Escherichia coli* from women with community-acquired lower urinary tract infections in 17 countries (ECOSENS project)

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Objectives: In the ECO.SENS project the prevalence and susceptibility of pathogens causing community-acquired acute uncomplicated urinary tract infections in women in 16 European countries and Canada was investigated (JAC 2003; 51: 69–76). It revealed vast differences in resistance between countries. We used the PhenePlate™ (PhP) System to determine the clonal diversity of the 2481 *E. coli* of the ECO.SENS survey.
Methods: The *E. coli* were typed using the PhP system. This is a biochemical typing method which measures not only positive and negative reactions but also the kinetics of each reaction. PhP-RE plates, specifically intended for use with *E. coli*, include 11 reagents. The speed and result of the reactions (measured at 8, 24 and 48 h of incubation) are expressed in a numerical code. Data were analysed with the PhPWIN Software.
Results: The PhP system divided the 2481 *E. coli* strains into 74 Common PhP Types (CT), each containing two or more isolates ($n = 2067$), and 414 Single PhP Types. The diversity index was 0.94 among all isolates. The antimicrobial susceptibility patterns identified 46 types and yielded a diversity of 0.55 among all isolates. Type CT-48 was the most frequent type in 13 countries and the second most frequent in four countries. It contained 400 isolates and exhibited 11 different antimicrobial resistance patterns. Sixty-four per cent of the *E. coli* isolates did not exhibit resistance to any of the investigated drugs. Frequent susceptibility patterns were isolated resistance to ampicillin, combined resistance to ampicillin and trimethoprim, single resistance to trimethoprim followed by single resistance to nalidixic acid.
Conclusion: Although some types exhibited distinct differences in their susceptibility patterns there was no obvious correlation between the phenotypes identified with the PhP system and the susceptibility pattern. Thus, our data did not suggest a dissemination of resistant clones within or between countries as an explanation for differences in antimicrobial resistance rates.

P1102 Characterisation of clinical isolates of Enterobacteriaceae resistant to third generation cephalosporins from a university hospital in Bratislava, Slovakia

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Objectives: A total of 101 clinical isolates of *Klebsiella pneumoniae* (67), *Escherichia coli* (14), *Enterobacter cloacae* (10) and *Citrobacter freundii* (10) resistant to third-generation cephalosporins (TGC) were isolated in Ruzinov University Hospital in Bratislava (Slovakia) during a discontinuous survey performed during a few months of 1998, 1999, 2001 and 2002. The aim of this study was to determine the mechanism(s) of resistance to TGC and to investigate the epidemiology of ESBL producers.
Methods: Susceptibility testing was performed by disk diffusion on Mueller–Hinton agar, and the double-disk synergy test (on medium with and without oxacillin) was used for detection of ESBL production. The determination of the pI of the β-lactamases was performed by isoelectric focusing. The relatedness of the isolates was investigated by pulsed-field gel electrophoresis (PFGE). One isolate of each pulsotype was used for analysis of the bla gene by PCR using primers specific for TEM, SHV and CTX-M enzymes.
Results: The double-disk synergy test was positive for all isolates of *K. pneumoniae*, 12 isolates of *E. coli* and only for two isolates of *E. cloacae* and two isolates of *C. freundii*. The isolates with negative double-disk synergy test were assumed to be mutants that overproduced their chromosomally encoded cephalosporinase. PFGE revealed respectively 10, 8, 7 and 5 pulsotypes among the isolates of *K. pneumoniae*, *E. coli*, *E. cloacae* and *C. freundii*. SHV-type ESBLs with pI 7.6 were found in all species, SHV-type ESBLs with pI 8.2 were present in isolates of *K. pneumoniae* and *E. cloacae*. TEM-type ESBLs with pI 5.2 and 6.0, as well as a CTX-M-type ESBL with pI 8.4 were produced by isolates of *K. pneumoniae*. Two strains of *K. pneumoniae* produced two different ESBLs simultaneously (one with a TEM-type of pI 6.0 plus SHV-type of pI 7.6, and one with a SHV-type of pI 8.2 plus a CTX-M-type of pI 8.4).
Conclusion: The production of ESBL was the major mechanism of resistance to TGC in Ruzinov University Hospital in Bratislava. At least five different ESBLs were encountered and a few clonal strains disseminated in different wards.

P1103 Role of clonal occurrences of multi-drug-resistance (MDR) in the MYSTIC Programme (USA; 1999–2003)

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Background: The Meropenem (MEM) Yearly Susceptibility Test Information Collection (MYSTIC) Programme was initiated in 1997, but in 1999 for the USA. This programme monitors resistance (R) in participant centres where carbapenems are prescribed and drug use data are obtained. An earlier report found antimicrobial use was not a clear cause of local or aggregate changes in R rates (Mutnick et al., JAC 2004). This study addresses the role of dissemination of R clones on R rates for non-fermentors *Acinetobacter* spp. (ACB) and *P. aeruginosa* (PSA).
Methods: Carbapenem (CARB)-multi-drug R strains (MDR) from among 226 ACB and 1112 PSA were tested by reference broth microdilution methods, automated ribotyping and PFGE to determine possible clonal dissemination. Each strain was also tested for metallo-beta-lactamases (MBL) and then analysed by CARB-R rate (phenotypic and PCR) and DDD/100 days use groupings (high, moderate, low).
Results: For the aggregate 15 sites in the MYSTIC Programme each year, the PSA CARB-R rate decreased over 5 years (16.1 vs. 7.3%); but other drug-R rates generally escalated. ESBL-R rates were stable in *E. coli* and *Klebsiella* (1–7%). Changes were not related to use calculations. Discovered clonally spread strains were elevated in high-R (1.8 clones/site) and moderate-R (0.6 clones/site) rate centres (22–30% of CARB-R strains were clonal), compared with unique MDR-PSA in low-R hospitals. ACB clonality was extreme in one geographic area with dissemination of five clones (931.7/B, C or D; 1090.2/A; 167.5/A) in four centres (02, 04, 06, 18). R-rates in ACB and PSA were clearly related to clonal occurrence and spread, and one MBL (VIM-7; Toleman et al., AAC 2004) was detected, representing its persistence in a Texas site. Decreased CARB-R rates from 1999 to 2002 were directly attributable to the disappearance of R clones in some locations.
Conclusions: ACB and PSA CARB-R and MDR-R rates in MYSTIC Programme institutions have been greatly influenced by clonal dissemination, less by antimicrobial use patterns. Most severe examples of clonality were observed among ACB in New York City and the documented endemic nature of VIM-7 PSA (0.9% of all PSA isolates). MEM remained the most active agent tested in the programme and surveillance networks must implement epidemiologic typing to assess the role of clonal spread on the R rates.

P1104 Optimising pharmacodynamic target attainment using the MYSTIC antibiogram (OPTAMA) – *Acinetobacter* spp. and *P. aeruginosa* in Europe, 2002

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Objectives: The goal of OPTAMA is to provide insight into the most appropriate antibiotic options for empiric therapy of common nosocomial pathogens. This is accomplished by considering the variability in pharmacokinetic (PK) parameter estimates, dosage regimens and MIC distributions from varying regions of the world to calculate the probability of attaining critical pharmacodynamic (PD) targets. For the 2002 data analysis Europe was divided into North (NE), South (SE) and East (EE) regions.
Methods: A 5000 subject Monte Carlo simulation was conducted to estimate PD target attainment (TA) for meropenem, imipenem, ceftazidime (CF), cefepime, piperacillin/tazobactam (PT), and ciprofloxacin (CP) against *Acinetobacter* spp. (AS) and *P. aeruginosa* (PSA). PD targets were free drug of 40%T > MIC for M and I, 50%T > MIC for CZ, CM and PT, and a total AUC/MIC ratio of 125 for CP. Standard dosing regimens for Europe were used. PK variability was derived from existing healthy subject data. MIC data were obtained from the MYSTIC Program.
Results: Probabilities of TA are listed in Table 1.

Table 1.

	Target attainment (%)					
	AS			PSA		
Drug regimen	EE	SE	NE	EE	SE	NE
Meropenem 500q8h	53.5	66.2	88.7	58.5	67.5	81.4
Meropenem 1000q8h	57.6	81.8	93.1	63.2	75.5	81.4
Imipenem 500q6h	54.2	73.0	94.6	56.9	64.9	80.5
Ceftazidime 1000q8h	22.1	22.4	70.7	53.7	69.8	78.5
Ceftazidime 2000q8h	29.2	37.7	79.1	59.6	79.5	84.3
Cefepime 2000q12h	34.7	53.9	72.9	55.0	62.7	81.0
Pip/Taz 4.5q8h	12.3	14.0	45.5	27.9	39.0	46.7
Pip/Taz 3.375q6h	16.1	19.6	–	37.3	55.9	–
Cipro 400q12h	14.1	10.7	40.2	28.1	22.8	39.1
Cipro 400q8h	17.9	15.7	52.3	37.8	31.0	47.7

Conclusions: NE shows the highest TAs and EE the lowest. Carbapenems show the best TAs achieved. Other β-Lactams, particularly PT and CF, do not possess good TAs. Desirable TAs are not readily attainable against either pathogen, even when doses are high. CP achieved the lowest TAs for both pathogens.

P1105 Optimising pharmacodynamic target attainment using the MYSTIC antibiogram (OPTAMA) – *Escherichia coli* and *Klebsiella pneumoniae* in Europe, 2002

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Objectives: The goal of OPTAMA is to provide insight into the most appropriate antibiotic options for empiric therapy of common nosocomial pathogens. This is accomplished by considering the variability in pharmacokinetic (PK) parameter estimates, dosage regimens and MIC distributions from varying regions of the world to calculate the probability of attaining critical pharmacodynamic (PD) targets. For the 2002 data analysis Europe was divided into North (NE), South (SE) and East (EE) regions.
Methods: A 5000 subject Monte Carlo simulation was conducted to estimate PD target attainment (TA) for meropenem, imipenem, ceftazidime, cefepime, piperacillin/tazobactam (PT), and ciprofloxacin (CP) against *E. coli* (EC) and *K. pneumoniae* (KP). PD targets were free drug of 40%T > MIC for M and I, 50%T > MIC for CZ, CM and PT, and a total AUC/MIC ratio of 125 for CP. Standard dosing regimens for Europe were used. PK variability was derived from existing healthy subject data. MIC data were obtained from the MYSTIC Program.
Results: Probabilities of TA are listed in Table 1.

Table 1.

	Target attainment (%)					
	EC			KP		
Drug regimen	East	South	North	East	South	North
Meropenem 500q8h	99.8	99.5	100	98.3	96.2	99.8
Meropenem 1000q8h	99.9	99.8	100	99.5	97.5	99.8
Imipenem 500q6h	99.2	99.7	99.9	99.3	97.1	99.8
Ceftazidime 1000q8h	80.5	95.1	96.5	52.0	83.1	88.3
Cefepime 1000q12h	78.5	98.8	99.7	67.4	96.0	98.7
Cefepime 2000q12h	84.0	100	99.8	77.0	96.0	99.4
Pip/Taz 4.5q8h	62.1	77.1	85.2	37.2	59.0	69.7
Pip/Taz 3.375q6h	70.4	87.4	92.1	45.1	72.9	81.2
Cipro 400q12h	57.7	62.7	80.9	60.8	69.1	72.4

Conclusions: NE shows the highest TAs and EE the lowest. Carbapenems show the most sustained high TAs, though in EE there is some reduction against KP. High TAs are more attainable against EC. Apart from in NE other β-Lactams, particularly PT, do not consistently possess high TA against EC and KP. CP achieved the lowest TAs for both pathogens.

P1106 Sustained activity of the carbapenems against Gram-negative pathogens: 6-year data from the UK MYSTIC (Meropenem Yearly Susceptibility Test Information Collection) programme

R. Masterton on behalf of the UK MYSTIC Group

Objectives: To monitor resistance trends to meropenem (MEM) and other broad-spectrum antibiotics over six years in UK centres through the MYSTIC Programme, a global, longitudinal antimicrobial surveillance study.
Methods: Isolates were tested using National Committee for Clinical Laboratory Standards methodology to determine the susceptibility breakpoints of MEM and several other antimicrobial agents including imipenem (IPM), ceftazidime (CAZ), piperacillin–tazobactam (TAZ), ciprofloxacin (CIP) and gentamicin (GM). 1136 Gram-negative isolates were collected from three centres in the UK from 1997 to 2002. Data are grouped in 2-year blocks.
Results: The study susceptibility results are shown in Table 1. Overall the carbapenems were the most active antimicrobial agents. They were also the most active against non-fermenters, including *Pseudomonas* spp. and *Acinetobacter* spp. With the exception of susceptibility to CIP, which decreased amongst Enterobacteriaceae at the end of the 6-year period all antibiotics tested retained their levels of activity. The proportion of extended-spectrum beta-lactamse (ESBL) and AmpC-producing enterobacteriaceae increased during the study (4.8 and 11.3% in 1997–1998; 7.4 and 16.7% in 2001–2001, respectively). Both MEM and IPM retained their potency against these ESBL and AmpC-producing isolates (100% for all time periods). All the other antimicrobial agents tested had much lower susceptibility against these resistant isolates and this decreased further over the 6-year period, with the exception of TAZ, which maintained its low levels.

Table 1. Percentage susceptibility of isolates over the study period

	MEM	IMI	CAZ	TAZ	CIP	GM
Enterobacteriaceae						
1997–1998 ($n = 186$)	100	99	80	75	94	90
1999–2000 ($n = 280$)	100	100	81	74	94	90
2001–2002 ($n = 270$)	99	98	83	71	85	89
Non-fermenters						
1997–1998 ($n = 94$)	86	90	70	85	70	75
1999–2000 ($n = 142$)	97	94	70	83	80	70
2001–2002 ($n = 128$)	90	87	71	87	77	74
All Gram-negatives						
1997–1998 ($n = 280$)	95	96	77	81	86	85
1999–2000 ($n = 422$)	99	98	77	83	89	83
2001–2002 ($n = 434$)	97	94	80	80	84	85

Conclusions: During this 6-year study, some minor but no major susceptibility trends were observed. Although all antibiotics tested retained acceptable activity the carbapenems remained the most active antimicrobial agents against Gram-negative bacteria, including ESBL and AmpC-producing isolates. Continued surveillance is needed to monitor future resistance trends.

P1107 *P. aeruginosa* clonal dissemination in Brazilian intensive care units during 2002

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Objective: To investigate the clonal dissemination of multiresistant *P. aeruginosa* causing nosocomial infections within and between Brazilian intensive care units, which participated in the MYSTIC Program Brazil 2002.
Methods: Thirty-six *P. aeruginosa* isolates with the same phenotypic characteristics were collected during 2002 at four centres in São Paulo (#1, 4, 6, 7) and at one centre in Brasília (#5). They were analysed by pulsed field gel electrophoresis (PFGE). Isolates resistant to meropenem or imipenem plus at least two of the following drugs: ciprofloxacin, cefepime, ceftazidime or piperacillin/tazobactam were studied. SpeI chromosomal restriction fragments were separated with CHEF-DR III System. Electrophoretic patterns were analysed with GelCompar II v. 2.5 (Applied Maths, Kortrijik, Belgium). Interpretative criteria used were those described by Tenover *et al.*
Results: Five major clones were identified (A, B, C, D, G). Clone A was constituted by eight samples with indistinguishable PFGE pattern present in two centres (#1 and 6). Clone A also had closely related strains (A1–3) present in three centres (#1, 5 and 6). Clone B was constituted by four indistinguishable samples predominant in centre 6. Clone C had three indistinguishable samples, with closely related clones (C1–3). Also, Clone D had three indistinguishable samples, with closely related (D1) and possibly related (D2/D3) clones. Clones C and D were present in centre 1, as well as other related clones (C1–3 and D1–3). Clone G was constituted by two indistinguishable samples and was present in centre 7. Finally, eight samples were not related. Centre 4 did not present any cloned isolates.
Conclusions: Clonal dissemination was detected within (Clones A, B, C, D, and G) and between centres (Clone A). These findings are important when analysing surveillance data, since susceptibility rates may be significantly affected with elevated resistance mainly due to clonality.

P1108 Resistance of *Pseudomonas aeruginosa*: 10-year experience in a Portuguese hospital

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Objectives: The aim of this study was to evaluate the evolution of antibiotic resistance pattern of *Pseudomonas aeruginosa* strains isolated at a central hospital in Lisbon for a period of 10 years.
Methods: This was a retrospective study of 2219 pathogenic strains isolated between 1993 and 2002. Bacterial identification was achieved using the APi system (BioMérieux) and the susceptibility tests were performed by the disk diffusion method of Kirby-Bauer according to NCCLS. Since 1994, bacterial identification and susceptibility tests were performed by Vitek system (BioMérieux). Gentamicin, tobramycin, amikacin, ceftazidime, ciprofloxacin, imipenem, aztreonam and the association piperacillin/tazobactam were tested.
Results: The 2219 pathogenic strains were distributed uniformly during the 10 years of study. The resistance rate to aminoglycosides reached its highest value in 1997 and its lowest in 2000. The resistance rate to gentamicin ranged from 12 to 46%; to tobramycin ranged from 5 to 27% and to amikacin ranged from 2 to 17%. In 1993, it was introduced as a new surveillance programme of pharmacokinetics control (Kinetidex) of the aminoglycosides starting with gentamicin and later with tobramycin and amikacin. The resistance rate to ceftazidime ranged from 6% (1995) to 35% (2001); to ciprofloxacin ranged from 10% (1995) to 42% (1998), both with progressive increase. The resistance rate to imipenem ranged from 8% (1995; 2000) to 27% (1999). The

resistance rate to aztreonam ranged from 18% in 1994–1995 to about 35% in the last 5 years, although it is not used in our hospital. The resistance rate to the association piperacillin/tazobactam was progressively increased, from 9% in 1993 to 30% in 2001–2002.
Conclusions: The resistance rates of *Pseudomonas aeruginosa* to ceftazidime, ciprofloxacin and piperacillin/tazobactam doubled in 10 years. The resistance rate to imipenem, even thought is a third-line choice antibiotic, has not increased. The resistance rates to the aminoglycosides had the highest value in 1997 but, with the implementation and the optimisation of the pharmacokinetics control programme, we have seen a decreasing pattern of resistance.

P1109 Convergent resistance development between two carbapenems for *Pseudomonas aeruginosa*

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Objectives: The objectives of this study were to evaluate the activity of meropenem against isolates of *P. aeruginosa* and to investigate the relationship between imipenem and meropenem in selecting resistance in *P. aeruginosa*. *P. aeruginosa* isolates ($n = 104$) were collected from Edinburgh between January and May 2003.
Methods: The MICs of imipenem, meropenem, ceftazidime, piperacillin/tazobactam and ciprofloxacin were determined by agar dilution according to the BSAC guidelines.
Results: Meropenem had the lowest level of resistance at 1.9%, followed by piperacillin/tazobactam with 4.8%, imipenem at 5.8%, ceftazidime with 13.5% and ciprofloxacin with 20% resistance. In order to assess any relationship between meropenem and imipenem in the selection of carbapenem resistance by *P. aeruginosa* the ratio of imipenem MIC to meropenem MIC was compared with the imipenem MIC for each strain. At low MICs, meropenem was commonly more than 10-fold more active than imipenem, but as the imipenem MIC increased, the ratio decreased. A similar analysis was performed on a random set of 342 strains from European MYSTIC data from 1997 to 2000 in which the same relationship occurred.
Conclusion: The results of both sets of analyses suggest that imipenem could be a more powerful selector of resistance than meropenem. However, in the strains analysed, once resistance has been selected, it conferred a similar degree of insusceptibility to both imipenem and meropenem. Therefore, in order to minimise the risk of multiple carbapenem resistance developing, the most active group member should be selected to treat infection with *P. aeruginosa*, this agent would be meropenem.

P1110 Lack of association between hypermutation and antibiotic resistance development in *Pseudomonas aeruginosa* from intensive care unit patients

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Objectives: Antibiotic resistance in *Pseudomonas aeruginosa* from cystic fibrosis (CF) patients has been found to be linked to the presence of hypermutable strains. The objective of this work was to study the role of hypermutation in antibiotic resistance development in patients from Intensive Care Units (ICU).
Methods: A total of 216 *P. aeruginosa* isolates recovered from clinical samples of 103 patients admitted to the H. Son Dureta ICU from September 2002 to November 2003 were analysed. Identification and initial susceptibility testing was performed with the WIDER system. Additionally, MICs for ceftazidime (CAZ), cefepime (FEP), imipenem (IMP), meropenem (MER), ciprofloxacin (CIP) and tobramycin (TOB) were determined with Etest. The first *P. aeruginosa* isolate obtained from each of the patients and type of clinical sample as well as any subsequent isolate that developed resistance to any of the antibiotics tested were further studied. Pulsed field gel electrophoresis (PFGE) and mutation frequencies (mf) estimations were performed in these isolates by standard procedures.
Results: Following the defined criteria 160 isolates from the 103 patients were included. With the exception of IMP primary resistance was low or moderate for all antibiotics: 7.5, 6.5, 23.4, 11.2, 8.4 and 0% for CAZ, FEP, IMP, MER, CIP and TOB, respectively. On the other hand, resistance development during antibiotic treatment (secondary resistance) had a high impact in resistance. *P. aeruginosa* strains from 20.4% of the patients developed resistance to at least one of the antibiotics (15.5, 17.5, 8.7, 8.7, 8.7 and 1% for CAZ, FEP, IMP, MER, CIP and TOB, respectively). PFGE revealed that most of the patients were infected with unique *P. aeruginosa* clones (82 different clones for the 103 patients). Also, when a subsequent resistant isolate was recovered from a given patient it was, in most cases, consequence of resistance development in the infecting strain and not of its replacement by a different resistant clone. Mutation frequencies estimations revealed that only one of the 160 isolates (<1%) was hypermutable (mf 6.0×10^{-6}) whereas the other 159 were non hypermutable (mean mf 1.8×10^{-8}).
Conclusions: Despite *P. aeruginosa* mutational antibiotic resistance development has an important impact in the ICU setting, selection of hypermutable strains is a very infrequent event, in contrast to what happens in CF patients.

P1111 Detection and susceptibility testing of hypermutable *Pseudomonas aeruginosa* strains with the Etest

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Objectives: Detection of hypermutable *Pseudomonas aeruginosa* strains from cystic fibrosis (CF) patients by microbiology laboratories might be useful for establishing adequate antimicrobial therapies due to the link between the presence of these strains and antibiotic resistance development. The objective of this work was to study the reliability of the Etest method for the detection and susceptibility testing of hypermutable *P. aeruginosa* isolates.
Methods: Strains PAO1 and its hypermutable derivative PAOdeltamutS were used for the standardisation of the procedure, which was tested with 35 *P. aeruginosa* isolates from 21 CF patients. Mutation frequencies were estimated by standard methods. MICs were determined using Etest strips for ceftazidime, imipenem, meropenem, ciprofloxacin and tobramycin. The presence (or absence) within the inhibition zones of resistant mutant subpopulations (RMS), as well as their relative numbers and their highest MICs were recorded.
Results: Of the 35 isolates 10 (29%) were found to be hypermutable by mutation frequency estimations. Although the observation of RMS within the inhibition zones of an individual antibiotic was only suggestive of the presence of a hypermutable strain due to the occasional documentation of RMS in non hypermutable strains, the presence of RMS with three or more antibiotics unequivocally identified the strains as hypermutable (10/10 vs. 0/25 for hypermutable and non hypermutable strains, respectively). Additionally, this method allowed us to differentiate a dual effect of hypermutation in antibiotic resistance: (i) hypermutable isolates were substantially more resistant than non hypermutable isolates, and (ii) the resistance of hypermutable isolates was dramatically increased due to the presence of RMS.
Conclusions: (1) the Etest can be used as a simple method for the detection and susceptibility testing of hypermutable *P. aeruginosa* strains. (2) This method allowed as to differentiate the dual effect of hypermutation on antibiotic resistance. (3) This differentiation might be relevant for the design of adequate treatments since, in

contrast to what happens with the first premise, the second can be overcome by antibiotic combinations. A guideline for the detection, susceptibility testing and reporting of hypermutable *P. aeruginosa* strains with the Etest is proposed.

P1112 Partial reduction of antimicrobial resistance of *Pseudomonas aeruginosa* following new antibiotic strategies in an intensive care unit in Italy

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Objective: To evaluate the impact of a strategy of rotating and choosing antibiotics on the basis of local patterns of antimicrobial resistance (AMR) and synergy assays, in patients with *Pseudomonas aeruginosa* suspected or confirmed infections.
Methods: During two consecutive periods we prospectively evaluated AMR patterns of *P. aeruginosa* isolates in patients admitted for more than 48 h to the intensive care unit (ICU) of Verona. Susceptibility tests were performed by Kirby Bauer method. Data refer only to strains with different antibiotype in case of repeated isolations in the same patient. We classified isolates as multidrug resistant (MDR) when resistance to piperacillin, imipenem, ceftazidime and gentamicin was detected and as PAN resistant (PAN-R) when the isolate was resistant to all tested antibiotics. Following the alarming observation of very high levels of AMR (Table) during the first year of study (Period A: April 2001 to March 2002), we modified the local protocols of empiric therapy recommending to possibly avoid the use of aminoglycosides and to use the association of a cephalosporin of third- or fourth-generation plus levofloxacin in severe nosocomial sepsis. Furthermore in infections due to MDR *P. aeruginosa*, we chose the most effective antibiotic combination on the basis of synergy assays (Etest). One year later (Period B: April 2002 to March 2003) we evaluated again AMR patterns in *P. aeruginosa* isolates.
Results: During period A, among 183 isolates of *P. aeruginosa*, 27 (14.7%) were MDR, of which 14 PAN-R. During period B (174 isolates), among 34 (19.5%) MDR strains, 11 were PAN-R. Comparing periods A and B, we observed an overall decrease of AMR levels of *P. aeruginosa* isolates against the principal antibiotics, which in particular was statistically significant for amikacin and ciprofloxacin (Table).

Anitbiotic	Period A (183 isolates) R %	Period B (174 isoloates) R%	*P*
Piperacillin/ tazobactam	(79) 43.2%	(67) 38.5%	NS
Gentamycin	(95) 51.9%	(85) 48.8%	NS
Ciprofloxacin	(103) 56.3%	(74) 42.5%	.013
Imipenem	(105) 57.4%	(87) 50.0%	NS
Amikacin	(61) 33.3%	(32) 18.4%	.002
Ceftazidime	(72) 39.3%	(68) 39.1%	NS
Cefepime	(68) 37.2%	(51) 31.3%	NS
Aztreonam	(97) 53%	(72) 46.4%	NS

NS: not significant

Conclusions: AMR of *P. aeruginosa* isolates remains an alarming issue in the studied ICU. In this setting the isolation of MDR and PAN-R strains was confirmed as a relatively frequent event during the last 2 years. Nevertheless the application of a new antibiotic strategy contributed to determine a relevant decrease of resistance levels against the principal antibiotics. Furthermore we believe that it is worth planning a case control study to evaluate the clinical efficacy of the combination of beta-lactams plus fluoroquinolones which demonstrated synergy in several *vitro* studies against MDR strains of *P. aeruginosa*.

P1113 Polyphasic approach to the characterisation of *Pseudomonas aeruginosa* isolates from a Portuguese central hospital

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Objectives: Bacteria can have population structures ranging from fully panmictic to highly clonal. Despite many studies, the population structure of *Pseudomonas aeruginosa* is still unclear. In this work a polyphasic approach was used based on two DNA-based fingerprinting methods and an antimicrobial resistance fingerprinting, which are combined using biological data analysis software in order to get an insight into the population structure of *P. aeruginosa* clinical isolates in a Portuguese Central Hospital.
Methods: A representative sample of *P. aeruginosa* clinical isolates (180/400) from different biological products/patients/wards was collected at a Portuguese Hospital during the year 2002. The strains were biochemically identified (Vitek auto microbic system). Two genomic typing systems, namely the minisatellite-primed PCR (MSP-PCR) and the enterobacterial repetitive consensus sequence PCR (ERIC-PCR), were used to discriminate the strains. The antibiotic susceptibility was analysed using the Vitek AMS System. The data obtained from DNA-based fingerprinting and antiobiotyping were combined and analysed using BioNumerics biological data analysis software.
Results: In the dendogram from the composite data set we identified a reduced number of genomic groups or clonal complexes (CCs) with >80% similarity. Most CCs contain strains from different origins, suggesting high rates of nosocomial migration and an outstanding versatility probably caused through recombination. The variability within each ward of the hospital was as nearly as within the whole population. Although some clusters showed >90% similarity, we also observed unique isolates, some of which diverged considerably from the rest of the population. The close genomic relationship among the isolates of each clone was detected by MSP-PCR analysis, pointing to its potential use in clinical settings to recognise epidemic *P. aeruginosa* clones over the short term. It also permits the selection of a few representative strains of each group for, as an example, antimicrobial chemotherapy studies.
Conclusion: There is sufficient justification to suggest that *P. aeruginosa* displays an epidemic population structure in this Hospital.
Acknowledgements: This study was supported by a research grant from Fundação Calouste Gulbenkian.

P1114 Emergence of *Pseudomonas aeruginosa* (PA) resistance to imipenem vs. meropenem

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Background: Resistance of *Pseudomonas aeruginosa* (PA) to antipseudomonal carbapenems is usually due to the combination of low intracellular concentrations due to OprD loss and efflux pumps and slow hydrolysis by beta-lactamases. For MER resistance to arise, both OprD loss and efflux pump expression is required, while IMP is affected only by OprD. Therefore it has been hypothesised that the two mechanisms required for MER resistance will result in lower likelihood of emergence of resistance.
Methods: 10 PA clinical isolates and one ATCC strain were studied. About 5×10^4 CFU/well were inoculated onto 96 multi-well plates containing Mueller–Hinton broth (MHB) serial dilutions of IMP and MER. Isolates were serially transferred. Number of transfers in sub-MIC concentration until emergence of resistance was determined. MICs were determined according to NCCLS guidelines. OMPs mapped on SDS-PAGE.
Results: Each of the selected isolates had distinct PFGE pattern. All had MIC <2 for ciprofloxacin, two were resistant to ceftazidime (CTZ). The MIC50 for IMP was 2 (range 1–4) and for MER 1

(range 0.25–2). Emergence of resistance was detected after two to eight passages and occurred at similar rate for both IMP and MER (average 4.6 vs. 4.8 passages). In each isolate resistance to both agents occurred at the same pace (up to one passage difference). At the time IMP resistance emerged, CTZ MICs did not change in seven strains and increased two to five dilutions in three strains. On emergence of MER resistance, CTZ MICs did not change in five isolates and increased two to four dilutions for the other five. Only one of these isolates became resistant to CTZ. Over-2-dilution-increase in ciprofloxacin or tetracyclin MIC occurred in one isolate at emergence of resistance to IMP and in six at emergence to MER. OMPs and beta lactamases activity correlates were determined.
Conclusions: We found similar pace of emergence of MER resistance compared with IMP in PA, despite the lower MIC, and the additional mechanism required for its development. The loss of OprD by both agents and induction of efflux pumps by MER were shown.

P1115 Risk factors for emergence of resistant *Pseudomonas aeruginosa* to beta-lactams in intensive care units

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Objectives: Emergence of *P. aeruginosa* resistance to antimicrobial agents is usual in intensive care units (ICU) and could be correlated with the use of some specific agents. We attempted to find a correlation between the use of various beta-lactams and the emergence of specific mechanisms of resistance.
Methods: We performed an open prospective study for a 3-year period including all patients for which *P. aeruginosa* was isolated from one or more specimens: bronchial aspiration, blood cultures, catheters, urinary cultures. Antibiotics we focused on were: amoxiclav, ticarcillin, piperacillin–tazobactam, cefotaxime, ceftazidime, cefepim and imipenem. Mechanisms of resistance we studied were: production of penicillinase or cephalosporinase, non-enzymatic mechanisms and loss of porine OprD2. Khi-2 test was used with a level of significance of 0.05.
Results: 132 patients were included in the study. Eighty-two strains emerged with mechanisms of resistance during antibiotic treatment. When using multivariate analysis no correlation was found between the use of amoxiclav, piperacillin–tazobactam, cefepim and any of the mechanisms. The use of cefotaxime was associated to the appearance of a non-enzymatic mechanism ($P = 0.005$). No correlation was found with the use of ceftazidime but if resistance appeared during ceftazidime treatment, it was due to secretion of cephalosporinase ($P = 0.001$). There was a significant relation between the use of imipenem and emergence of resistance by loss of porine OprD2 ($P = 0.00001$). Thirty-six strains from nine patients, analysed with pulsed-field gel electrophoresis, show that for a same patient all the strains were genetically linked together.
Conclusion: Our results show a high risk of selection of non enzymatic resistance by cefotaxime and emergence of resistance during treatment with imipenem.

P1116 Molecular genotyping of *Pseudomonas aeruginosa* clinical isolates resistant to quinolones: detection of mutations in the gyr genes

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Objectives: The aim of this study is to assess the diversity and genomic variability of quinolone resistant *Pseudomonas aeruginosa* isolates from 35 patients from a ICU of a hospital from the central part of Portugal. A second objective of the present study was to examine the mutations in the quinolone resistance determining regions (QRDR) of the gyrA, gyrB, parC and parE.
Methods: Bacterial isolates were identified using the VITEK System (Biomerieux). Their antibiotic resistance pattern was also analysed by MIC determination to several antimicrobial agents. Quinolone resistant *P. aeruginosa* isolates were used for following studies: The bacterial isolates were analysed by several molecular typing methods namely, Rep, Box and ARDRA PCR, in order to select those methods that will be reliable proof for epidemiologic studies. Due to its discriminatory abilities pulsed field electrophoresis (PFGE) and ribotyping techniques were also used. Single cell conformation polymorphism technique (SSCP) complemented with DNA sequencing (automated) were used in order to detect the mutations in the gyr genes.
Results: The molecular techniques used for typing *P. aeruginosa* isolates proved to be very useful. An attempt was made to compare the patterns obtained by the different PCR based techniques as a rapid tool with prognostic value for epidemic and nonepidemic studies. PFGE proved to be the most powerful technique to achieve this objective. Mutation detection SSCP analysis allowed us to separate the 35 isolates in various different groups that after DNA sequencing proved to contain mutation in the QRDR region. In codon 464 of the QRDR region a change of a Serine (TCC) to a tyrosine (TAC) was detected. Previously a mutation on the same codon was described but instead of a tyrosine a phenylalanine (TTC) was inserted. The mutation detected by us in our isolates was described for *Salmonella enterica*, but not in *P. aeruginosa*. So far this seems to be the first time that this mutation is described in *P. aeruginosa*. Several silent mutations were detected in the QRDR region that apparently has no significant value for quinolone resistance.
Discussion: PFGE, although a very tedious technique, proved to be the most reliable and reproducible method to be used in epidemiologic studies. In the present study, by SSCP, some mutations were detected in the gyrA and gyrB genes of *Pseudomonas* strains. One of these mutations was not described so far and it seems that it could be assigned as a mutation implied in the quinolone resistance of the referred strain. WAVE DHPLC is a finer technique that is currently used for the detection of these mutations, but this technique could not be used for the present study. As the number of strains analysed was not excessive, we decided to complement SSCP results with DNA sequencing of this QRDR regions. Also, we are aware of the fact that quinolone resistance can also be due to multidrug efflux pumps (MDR). Our aim is to proceed with this study looking closer to other mechanisms implied in quinolone resistance in *P. aeruginosa*.

P1117 *In vitro* effects of silicone oil on the growth and adherence of *Pseudomonas aeruginosa*

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Objectives: Silicone oil has been used as an effective long term tamponade in vitreoretinal surgery. Bacterial endophtalmitis may occur in an eye after vitrectomy and silicone oil injection. Although postsurgical endophtalmitis is rare; it is the most serious complication of vitreoretinal surgery. *Pseudomonas aeruginosa* is one of the common causative agents of endophtalmitis. Bacterial virulence reflects the ability of infecting microorganisms to produce pathological effects in an invaded host. The aim of this study is to investigate the *in vitro* effects of silicone oil on the growth and adherence of *P. aeruginosa*.
Methods: *P. aeruginosa* ATCC 15442 strain was used in this study. Cell suspensions of bacteria were prepared from overnight culture on blood agar at 37 °C. Following preparing the bacteria suspensions, 1 mL of the bacteria suspensions (McFarland 1) were added to 9 mL silicone oil. Distilled water (DW) was used as a negative control and brain heart infusion broth (BHI) was used as a positive control. As examining that bacterial suspension formed a vacuole in the silicone oil, inner and the outer parts of the vacuole were investigated separately. The colony counts and number of bacteria adhered to buccal epithelial cells (gram stained) were determined daily, until no growth was seen in the sample taken from silicone oil.

Results: *P. aeruginosa* showed an apparent decrease in colony counts following 1 day after inoculation in inner part of the vacuole of silicone oil and were eliminated from the medium at the 14th day; in the outer part of the vacuole eliminated after the fifth day. Colony forming units of *P. aeruginosa* in the distilled water; peaked at the third day and began to decrease at the 14th day, but never eliminated through the 21st day like BHI broth. We observed that silicone oil also inhibited the adherence to buccal epithelial cells comparatively with BHI and DW.
Conclusion: Silicone oil, a retinal tamponading agent, has an antimicrobial activity against *P. aeruginosa*; this effect may be correlated with the exception of nutrients for the growth and the high surface tension of silicone oil that destroys the cell wall characteristics of microorganisms.

P1118 Susceptibilities of clinical *Acinetobacter baumannii* and *Stenotrophomonas maltophilia* isolates to 14 antimicrobial agents: results of the Antimicrobial Resistance Surveillance Study of the Paul Ehrlich Society for Chemotherapy, 2001

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Objectives: *S. maltophilia* and *A. baumannii* are important pathogens causing nosocomial infections, particularly in the compromised host. Intrinsic resistance to many antibiotics coupled with acquired resistance in both species represents a major clinical problem. The objective of this study was to evaluate the *in vitro* susceptibilities of contemporary clinical isolates to selected antibiotics.
Methods: In November 2001, a total of 158 isolates of *A. baumannii* and 183 isolates of *S. maltophilia* were prospectively collected from 26 Clinical Microbiology Laboratories distributed throughout three Central European countries (Austria, Germany, and Switzerland). Minimal inhibitory concentrations of antimicrobial agents (belonging to seven different drug classes) were determined using the broth microdilution procedure according to the standard of the German DIN: piperacillin, piperacillin–tazobactam, cefepime, ceftazidime, imipenem, meropenem, amikacin, gentamicin, tobramycin, ciprofloxacin, levofloxacin, moxifloxacin, co-trimoxazole, and doxycycline.
Results: One hundred and twenty-three and 173 isolates were collected from patients in intensive care units (ICUs) and non-ICU inpatient areas, respectively. The remainder were either from outpatients or data were not available. Susceptibility patterns of *A. baumannii* isolates showed the highest susceptibility to meropenem (97%) followed by imipenem (96%), doxycycline (91%), and tobramycin (90%). Ciprofloxacin, cefepime, ceftazidime, piperacillin and piperacillin–tazobactam were each active against <80% of isolates. Eight (5.1%) strains were resistant towards antibiotics of five or more drug classes. Levofloxacin (87%), co-trimoxazole (80%), and moxifloxacin (79%) were the most active compounds against *S. maltophilia*. The vast majority of isolates was resistant to β-lactams and aminoglycosides. One-third of *S. maltophilia* isolates displayed resistance towards five or more drug classes.
Conclusion: Carbapenems seem to be the most active antimicrobial agents against *A. baumannii* isolates recovered from patients in hospitals located in Germany, Austria, and Switzerland. *S. maltophilia* showed the highest susceptibility rates for levofloxacin and co-trimoxazole.

P1119 Resistance rates of *Pseudomonas aeruginosa* isolates using The Surveillance Network (TSN) Database – Greece

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Objectives: *Pseudomonas aeruginosa* (PA) isolates from nosocomial infections are frequently resistant to many antimicrobial agents. This study was designed to define the current incidence of antibiotic resistance of PA isolates to ceftazidime (CAZ) and ciprofloxacin (CIP), two drugs used broadly as first choice therapeutic options in serious infections.
Methods: TSN was used to analyse the antimicrobial resistance profile of non-repeat PA isolated from patients of two tertiary care Greek hospitals located at distinct areas during a period of 3 years (January 2000 to December 2002). Susceptibility data from isolates originating from patients on the intensive care unit (ICU) and inpatients (non-ICU) were compared. All PA included in the study were identified and tested for susceptibility using the VITEK system (bioMerieux, France). Susceptibility data were interpreted using NCCLS breakpoint criteria.
Results: During the 3-year time period data from 740 PA strains from ICU patients and 949 strains from inpatients were reviewed. Resistance rates for ICU to CAZ and CIP were 38.2 and 67.2% respectively, while for inpatients 17.4 and 37.1%, respectively. For both CAZ and CIP there was a statistically significant difference among ICU patients and inpatients ($P < 0.001$). For ICU patients there was a significant ($P < 0.001$) increasing annual trend of resistance towards both CAZ (34.4–48.5%) and CIP (62.3–79.1%). For inpatients a similar significant ($P < 0.01$) annual trend of resistance was observed for CAZ (13.5–23%), while for CIP there was no significant difference.
Conclusions: The high rates of antimicrobial resistance of PA to CAZ and CIP indicate that the broad use of these drugs needs to be revised and alternative therapies should be considered. Continuous surveillance studies in order to observe changes in resistance which might effect therapeutic choices are necessary.

P1120 Integrons carried by Enterobacteria from avian cloacal swabs harbour the same cassette arrays as isolates from clinical human origin

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Objectives: Gull populations have increased in many European towns during the past years. The proximity of these birds, which are able to nest on roofs and feed of urban waste, to human activities, together with their high power of dispersal, have risen the problem of their potential role in the dissemination of resistance genes. As many resistance determinants are inserted into integrons, we decided to investigate the frequency of integron carriage among Enterobacteria isolated from gulls and to compare their cassette content with that of integrons from human clinical isolates.
Methods: Enterobacteria were isolated from *Larus cachinnans michahellis* cloacal swabs and identified as *E. coli* ($n = 84$), *Proteus* sp. ($n = 89$), *Klebsiella* sp. ($n = 18$), *Salmonella* sp. ($n = 17$), *Citrobacter* sp. ($n = 6$) by conventional methods. Eighty epidemiologically unrelated clinical isolates (46 *Proteus* sp. and 34 *E. coli*), collected in the same period from the Trieste city hospital were also analysed. All strains were screened for the presence of the IntI1 gene by dot blot hybridisation of genomic DNAs. Positive strains were further analysed by amplification and characterisation of the integron variable regions. Selected *E. coli* strains of human and avian origin were typed by PFGE analysis of XbaI generated genomic DNA fragments.
Results: 25/214 (12%) avian enterobacterial isolates and 16/80 (20%) human clinical isolates carried the intI1 gene. Amplification and subsequent restriction analysis of the variable regions of class 1 integrons revealed the presence of 13 different integron types among the avian isolates, some of which were carried by isolates of different bacterial species and collected from different birds. Three of these integron types were recovered also from human clinical isolates. Nucleotide sequencing of the variable regions of these integrons revealed the presence of the following cassette arrays: InA: aadA1; InB: dhfrI, aadA; InC: dfr17, aadA5. Typing of six *E. coli* strains carrying InC, four of which collected from human blood specimens and two from birds, demonstrated no similarity of PFGE patterns among human and avian strains.
Conclusion: Our study demonstrates that resistance determinants carried by integrons are frequently present in avian strains. The

same integrons are also associated to clinically relevant, genotypically distinguishable human strains, thus supporting the hypothesis that integron carried resistance determinants are exchanged between gulls and humans.

P1121 Study of an imipenem-resistant *Klebsiella pneumoniae* cluster in a Greek hospital

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Objectives: Metallo-beta-lactamases (MBLs) producing bacteria present a major nosocomial infection risk and increased cautiousness is required to limit their spread.
Methods: In 'Sotiria' Chest Diseases Hospital of Athens six *K. pneumoniae* imipenem-resistant strains were isolated from patients hospitalised in ICU, from June 2003 to August 2003. The susceptibility phenotype was determined by the standard disk diffusion method (Kirby-Bauer) and the MIC by the automated system Vitek2 (Biomerieux, France). From the patients studied, four of six originated from hospitals other than ours and all were hospitalised in the ICU for separate reasons, but they all progressively developed VAP. Regarding the site of isolation, four of six strains were isolated from central venous catheters, two of six from bronchial secretions and one of six from urine sample of a patient who developed urine infection besides the VAP. The patients were treated with wide range antimicrobial chemotherapy, including third-generation cephalosporins, aminoglycosides and quinolones, with a favourable outcome. The imipenem-resistant *K. pneumoniae* strains were further investigated for MBL production, by the imipenem + EDTA disk synergy test se well as the MBL Etest with strips containing imipenem/imipenem + EDTA (AB Biodisk, Sweden).
Results: Positive results were obtained for all six strains, by conventional methods, indicating the MBL production. Molecular analysis proved that all six imipenem-resistant *K. pneumoniae* strains carried the blaVIM gene, while the ERIC-PCR revealed genotypic similarity among them, as well as among imipenem-resistant *K. pneumoniae* strains isolated in other hospitals in the capital of Athens.
Conclusion: The implementation of measures of increased caution and care in our hospital resulted in no further isolation of MBL-producing *K. pneumoniae* so far in the ICU.

P1122 Epidemiology of *Enterobacter aerogenes* in Belgium: preliminary results of a multicentre survey

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Objectives: A national prospective study was conducted in 2003 to characterise the epidemiology of *E. aerogenes* (EA) clinical isolates from patients in Belgian hospitals and to compare results with data of a similar survey in 2000–2001.
Methods: Antimicrobial susceptibility [MICs of piperacillin-tazobactam (PTZ), ceftazidime (CTZ), cefotaxime (CTX), cefepime (FEP), imipenem (IMP), meropenem (MER), gentamicin (GEN), amikacin (AMK) and ciprofloxacin (CIP) determined by the Etest method] and ESBL production (detected using the ceftazidime/clav and cefotaxime/clav Oxoid combination disks) were determined on strains collected from hospitalised patients (five consecutive non-duplicate EA strains for each centre) from January to July 2003.
Results: 87 centres (regional distribution as follows: 42 from Flanders, 30 from Wallonia and 15 from the Brussels area) sent 403 strains isolated from patients (mean age 69 years, range 1–98) hospitalised mostly in medical (29%), intensive care (22%), long-term care (16%) and surgical (13%) units. Strains mainly originated from urinary (39%) and respiratory (31%) tracts, wound swabs (11%) and blood cultures (4%). Eighty-five per cent of the strains were resistant to CIP, 78% to CTZ, 59% to PTZ, 18% to CTX, less than 9% to aminoglycosides and 1% to FEP and carbapenems. ESBL production was detected in 58% of the strains; these strains were more resistant to CTZ (100 vs. 47%; $P < 0.001$) and CIP (99 vs. 66%; $P < 0.001$) than non-ESBL-producing strains. The evolution of resistance rates between 2000–2001 and 2003 were compared in the 42 hospitals which took part in both studies. Strains were more frequently susceptible in 2003 to CTZ (23 vs. 14%; $P = 0.01$), CTX (70 vs. 45%; $P < 0.001$), FEP (99 vs. 91%; $P < 0.001$) and AMK (92 vs. 85%; $P = 0.04$), probably because strains showed a trend to be less frequently producers of ESBLs than in 2000–2001 (54 vs. 61%, $P = 0.17$).
Conclusions: These data confirm that multi-resistant EA strains are widespread in all care departments of Belgian hospitals but that the increasing prevalence of ESBL-producing strains seen in the past years appears to have more recently stabilised.

P1123 Antibiotic susceptibility in invasive *E. coli* isolates, Belgium, 2002

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Objectives: To monitor reduced susceptibility (RS) in invasive *E. coli* isolates in Belgium using validated antibiotic susceptibility testing (AST) results from diagnostic laboratories.
Methods: In October 2001, 27 diagnostic laboratories started to report AST results and extended spectrum beta-lactamase (ESBL) production in each first *E. coli* blood or cerebrospinal fluid isolate per patient per quarter, using their routine laboratory methods. Suspected ESBL positive strains were sent to the National reference laboratory. Quality assessment (QA) of AST in 2002 was done by sending two blinded *E. coli* strains of the European Antimicrobial Resistance Surveillance System (EARSS) to 107 laboratories. As defined by EARSS, isolates lacking mandatory information and duplicates within the same quarter were excluded. Statistical significance was tested using chi-square.
Results: In 2002, 1185 *E. coli* strains from blood or cerebrospinal fluid were reported. The mean age of patients was 67 years (range: 0–98) and 57% were female. RS was 48.5% for amoxicillin or ampicillin, 6.0% for gentamicin or tobramycin, 3.1% for third generation cephalosporins and 13.3% for ciprofloxacin or ofloxacin. ESBL production was detected in 19 (1.9%) of 983 isolates tested. RS to three or four of antibiotic classes was observed in 5.7% of the strains, while 47.7% were susceptible to all tested antibiotics. Of the 93 Belgian laboratories returning results for both blinded QA strains, 99% obtained correct identification and 92% correct AST results for all antibiotics included in the surveillance. The national reference laboratory received 15 of the 19 isolates suspected to be ESBL positive and confirmed all 15 cases.
Conclusion: In comparison with EARSS results for neighbouring countries, Belgium had a lower RS rate to aminopenicillins than France (57%; $P < 0.001$), a higher RS rate to third-generation cephalosporins than France, The Netherlands and Germany (respectively, 2, 1 and 1%; $P < 0.05$) and a higher RS rate to fluoroquinolones than France and The Netherlands (respectively, 9 and 6%; $P < 0.05$). Multiple drug resistance and ESBL production in Belgium, although still relatively low, certainly warrant monitoring because of a possible rapid increase through selection of linked resistance and spread and evolution of ESBL genes.

P1124 Dynamics of ciprofloxacin-resistance for Gram-negative bacilli isolated in urinary paediatric infections

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Objectives: To evaluate ciprofloxacin (CIP) resistance in urinary paediatric pathogens for years 1999 and 2002; to compare the results obtained.

Methods: The study was conducted on 698 strains (1999: 349; 2002: 349) isolated from urine, in children hospitalised in the 'Sf. Maria' paediatric hospital from Iasi, Romania, the largest paediatric hospital in eastern Romania. Identification was done by classical methods, and susceptibility testing by disk diffusion method (Bauer-Kirby) according to NCCLS criteria and controls. Thirty-six strains were tested by Etest (AB Biodisk, Solna, Sweden).
Results: In 1999, the resistance ratio was 9.16%, with *Pseudomonas aeruginosa* (85.7%), *Enterobacter cloacae* (37.5%), *Proteus mirabilis* (9.5%) and *Escherichia coli* (7.6%) on the first places. For 2002, the results were predictable, with an increase in resistance ratio to 14.8%, with the same pathogens as leaders: *P. aeruginosa* (55.15%), *E. cloacae* (46.15%), *K. pneumoniae* (15.5%) and *E. coli* (10.2%). Minimum inhibitory concentration determination proved high level of resistance (>32 μg/mL) for 89.4% of strains.
Conclusion: This survey – performed on strains isolated from urinary paediatric infections – shows an increase of resistance ratio in 2002, 14.8% compared with 9.16% in 1999. This behaviour is explained by the unrestricted use of CIP in therapy and sometimes in sequential schema for relapsing paediatric urinary tract infections. The results are an advertisement for clinicians that should reconsider the therapeutic option.

P1125 Antimicrobial resistance of *Escherichia coli* isolated in bacteraemia during 2002: results from the Ile de France Microbiologist Network

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Objectives: To survey antimicrobial resistance in *E. coli* bacteraemia in order to review chemotherapy guidelines.
Methods: All bacteraemia from eight French non-teaching hospitals around Paris were included. Community acquired (CA) or hospital acquired (HA) bacteraemia was based on patient history; sources were investigated clinically and microbiologically: urinary (U), gastrointestinal (GI), intravascular device (IVD), respiratory (R), surgical site (SS), foetomaternal (FM), others. Resistance was defined according to the recommendations of 'Comité de l'antibiogramme de la Société Française de Microbiologie' for amoxicillin (AMX), AMX + clavulanate (AMC), cefotaxime (CTX), gentamicin (GM), amikacin (AN), nalidixic acid (NAL), ciprofloxacin (CIP). Resistance rates, defined in accordance with ONERBA recommendations (www.onerba.org), were stratified by age, wards, sources.
Results: 1731 bacteraemia occurred in 1661 patients, 615 (36%) were *E. coli* bacteraemia (incidence: 0.75/1000 days of hospitalisation and 0.4/100 admissions) and 27% were HA bacteraemia. More than 50% of *E. coli* was isolated in patients older than 60 years. Sources were: U = 387 (63%), GI = 97 (16%), R = 15 (2.5%), FM = 11 (1.8%), SS = 10 (1.6%), IVD = 5 (0.8%), others = 90. Resistance rates are presented in the following table:

	% *E. coli* resistance (R+I)						
	n	AMX	AMC	CTX	GM	NAL	CIP
CA	445	56.4	49.1	0.9	1.1	10.7	6.2
HA	170	62.9	53.3	1.8	4.2	19.8	10.8
U CA	310	61.6	50	0	0.3	8.4	4.8
U HA	77	68.6	59.7	0	7.8	22.1	14.3
GI CA	73	49.3	45.2	1.4	1.4	15.1	8.2
GI HA	24	62.5	54.2	4.2	4.2	25	8.3

Resistance of *E. coli* HA to betalactams or quinolones in surgery was higher than in medecine (AMX: 64.9/55.1%, NAL: 25.8/17.4%, CIP:12.9/8.7%). A high rate of resistance to aminosides was observed among NAL R strains.
Conclusion: Yearly survey of severe infections like bacteraemia allows to up date chemotherapy guidelines: third-generation cephalosporins and gentamicin represent actually our basis of first-intention chemotherapy.

P1126 Colonisation of long-term-care facilities residents with antimicrobial-resistant enterobacteriaceae (EB) in Greece

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Objectives: Interfacility transmission of antimicrobial-resistant (R) pathogens has been well documented. Considering the increasing frequency of isolation of EB-R in hospitals, a survey was performed to determine the prevalence of LTCF residents colonised with EB-R.
Methods: 18 LTCF were randomly selected from the public sanitation list of Attic province. Urine, nasopharyngeal and wound samples were collected from 561 residents; from each LTCF, we chose randomly 30% of the existing population (minimum sum 25 residents). MacConkey agar plates were inoculated and colonies grown were identified by API strips (bio Mereux Vitek Inc) and underwent antimicrobial disk susceptibility testing, following the NCCLS guidelines. Confirmation of ESBL-R was done by MIC broth microdilution and double disk diffusion, as per NCCLS.
Results: 295 EB were isolated from 1148 samples (34.8%). Resistance to ampicillin (AMP), ampicillin–sulbactam (AM/SB), aminoglycosides (AMS), fluoroquinolones (FQ) and third-generation cephalosporines (Chep3) was analysed. AMP and AM/SB resistant rates were 67% (leading bacteria *Proteus* spp. 67% and *Escherichia coli* 53%) and 39% (leading isolates *Klebsiella* spp. 59% and *Proteus* spp. 46%), respectively. About 7.8% of the isolated EB were Chep3-R (*Proteus* spp. 17.6%, *Klebsiella* spp. 7.3% and *E. coli* 4.8%). About 48% of them were producing ESBL (leading isolates *E. coli* 50% and *Klebsiella* spp. 39%). About 62.5% of the ESBL strains were FQ-resistant. From AMS-R isolates (R-rate 22%), we distinguished the R-rates of *Providencia stuartii* (68%) and *Proteus* spp. (24%). R to FQ estimated to be 16% (leading isolates *Morganella morganii* 41% and *E. coli* 20%).
Conclusions: (1) Resistance among EB to Chep3, FQ and AMS in Greek LTCF is worrisome. (2) The close relationship between FQ-R and ESBL production is remarkable. (3) Resistance of *E. coli* to FQ (20%) is relatively high and must be considered, especially in case of urinary and respiratory infections.

P1127 Comparative evaluation of uropathogenic *E. coli* resistance during a 5-year period

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Objectives: To study the antimicrobial resistance of uropathogenic *Escherichia coli* strains (UPEC) among outpatients and inpatients hospitalised in different departments, during a 5-year period.
Methods: From 1998 to 2002 a total of 2983 *E. coli* strains were studied. They were isolated from urine cultures obtained from patients (adults and children) hospitalised or visited the outpatients clinics with clinical manifestations of urinary tract infection. The cultures were performed by conventional methods and the identification of *E. coli* was based on the API 20E system (BioMerieux). The susceptibility testing was carried out by the disk diffusion method (Kirby-Bauer) according to the current NCCLS guidelines. The expression of ESBLs was detected by the double disk synergy test in all strains isolated during 2002. The data analysis was based on WHONET software.

Results: The resistance rate (%) of 1998 and 2002 are presented in the table below:

Antibiotics	Ampicillin		Amox/ Clav		Cephalo-thin		Cotrim-oxazole		Norfloxa-cin	
Year	98	02	98	02	98	02	98	02	98	02
Internal Medicine Dept. $n = 1431$	39	39.6	8.4	4.5	9.9	14.9	25.8		4.5	11
Surgical Dept. $n = 528$	48.6	42.6	11.4	1.5	18.1	21.2	36.6	21.1	1.7	10.1
Out-patients $n = 596$	25.3	30.7	2	2.1	6.1	5.8	18.6	13	2.2	0
Children $n = 428$	43.8	44.6	3.7	2.9	6.1	5.8	25	14.3	1.5	1.1

The incidence of resistance to gentamicin (GEN), cefotaxime (CXT) and ceftazidime (CAZ) in 1998 and 2002 was as follows: GEN 1.6%/3.3%, CXT 0%/5.2%, CAZ 0.4%/2.4%. The resistance to imipenem and piperacillin/tazobactam was very limited (<1 and <2%, respectively). No significant increasing trends were observed during the study period for all antibiotics tested except from norfloxacin in hospitalised patients. During 2002, 3.8% of the strains isolated from hospitalised patients and 1.9% of those recovered from paediatric patients were ESBL-producing ones.

Conclusion: The resistance of UPEC is similar to those reported by other hospitals in our country and seems to remain stable during the last 5 years. The high incidence of resistant strains in paediatric patients reflects the prescription habits. The updated local surveillance data provide useful information for a successful empiric therapy.

P1128 Prevalence and antibiotic resistance of the most important urinary pathogens responsible for non-complicated cystitis in women: the IceA 2 Study

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Objectives: This observational study has set the aim of providing data both about the prevalence of the most important pathogens responsible for non-complicated acute cystitis in women and about resistance patterns to the most common antimicrobial agents. A female population representative of the national distribution with an evident pathology with related clinical and microbiological signs was evaluated.

Methods: Fifty researchers operating in Italy were chosen by the European School of General Medicine and enrolled. A broth dilution method using sensititre plates was performed as susceptibility test.

Results: 522 outpatient women (age: 18–57; average age: 35.7) with diagnosis of first episode of non-complicated acute cystitis were enlisted. Symptoms appeared between 24 and 48 h in 35.5%, 48–72 h in 27.1%, within 24 h in 26.7% of cases, in 10.7% symptoms duration was unknown. Bacteriuria was considered significant when concentration of one isolate was >105 CFU/mL. Two hundred and seventy-four specimens were positive and were processed, according to standard bacterial isolation and identification methods and 247 microorganisms were isolated. *Escherichia coli* (71.7%), *Klebsiella pneumoniae* (8.9%), *Proteus mirabilis* (8.1%) were the most frequent isolated uropathogens. The *in vitro* susceptibility of 169 *E. coli*, 22 *K. pneumoniae* and 20 *P. mirabilis* was tested against ciprofloxacin, levofloxacin, norfloxacin, fosfomycin/trometamol (FOS), nitrofurantoin (NIT), co-trimoxazole (SXT), amoxicillin/clavulanate (AMC). Against all the fluoroquinolones *E. coli* showed a susceptibility rate of 96.4%, *P. mirabilis* and *K. pneumoniae* of 100%. The susceptibility rates were 98.2, 70 and 77.3%, respectively, for FOS and 92.3, 100 and 95.4%, respectively, for AMC. SXT (82.4, 70 and 72.7%, respectively) and NIT (95.3% of susceptibility for *E. coli* and 59.1% for *K. pneumoniae*) showed good activity only against *E. coli*.

Conclusion: This epidemiological study showed that in Italy the aetiology of non-complicated acute cystitis (over of 50% of cases showed significant bacteriuria) is highly predictable and mostly associated with *E. coli*, *P. mirabilis* and *K. pneumoniae*. Fluoroquinolones still represent a rational choice confirmed by their microbiological effectiveness against the predominant pathogens. Ciprofloxacin retains excellent activity and is an appropriate choice for empirical therapy of UTIs because of its low possibility to create resistance, low rates of clinical failure and rapid symptom relief.

P1129 The prevalence of antibiotic resistance of *Escherichia coli* and Enterococci isolated from faeces of pigs in communal farming areas of Mafikeng district (R.S.A.)

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Aims and objectives: The aims of this study were to isolate *Escherichia coli* and Enterococcus from faeces of pigs, to determine their prevalence of antibiotic resistance and to check the presence or absence of vancomycin resistant Enterococci.

Methods: A total of 100 faecal samples were collected using sterile gloves directly from the rectum and put on sterile containers. Bacterial isolation were achieved by culturing approximately 1 g of faeces into 3 mL of nutrient broth and incubated for 24 h at 37 °C. Subcultures were performed on Eosin methylene blue agar and Enterococcus selective agar for *E. coli* and enterococci, respectively, and incubated for 24 h at 37 °C. Gram staining was performed, then API 20E and API 20STREP employed for *E. coli* and enterococci, respectively. Serological typing was performed on all *E. coli* species. Antimicrobial susceptibility test was performed on Mueller–Hinton Agar (Bauer et al., 1966) and blood agar using different antibiotics for *E. coli* and Enterococcus, respectively.

Results: Multiple antibiotic resistance was observed in most of the *E. coli* isolates, with *E. coli* O26:B6 having the highest incidence at 53.1% as compared with *E. coli* O111:B4, *E. coli* O126:B16 strains and other *E. coli* strains that did not react with any of the antisera used at 50, 46.7 and 18.2%, respectively. Multiple antibiotic resistance was also determined on Enterococcus species with *E. faecium* having the highest incidence at 85.7% as compared with *E. durans*, *E. avium* and *E. gallinarum* at 69.2, 61.9 and 60.2%, respectively. Despite the high incidence of antibiotic resistance, all Enterococccus isolates were susceptible to amoxicillin and vancomycin. The difference in the number and types of microorganisms in the faeces among the treatment group were determined by ANOVA and Duncan's multiple range tests using Minitab Release 13.1 software. There were significant differences among groups in microorganism's multiple antibiotic resistance, and resistance was determined by Chi-square. A significant value was determined at $P < 0.05$.

Conclusion: This study showed that vancomycin resistant Enterococci is not a problem in communally reared pigs, except for multiple antibiotic resistance.

S. pneumoniae: resistance and epidemiology

P1130 Antimicrobial resistance with *Streptococcus pneumoniae* in 2003 – results of the Multinational GRASP Surveillance Program

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Objectives: A multinational surveillance study, GRASP, was conducted between November 2002 and April 2003 with the aim of assessing rates of antimicrobial resistance among isolates of *Streptococcus pneumoniae* (Spn) in 20 countries in Europe, Eastern Asia and South Africa.
Methods: A total of 2487 isolates of Spn from patients with respiratory tract and systemic infections were characterised in a central laboratory (UIHC). MICs were determined by broth microdilution using methods described by the NCCLS. Specimen sources of isolates were: sinus aspirates and middle ear fluid, 27.6%; lower respiratory tract, 46.5%; blood cultures, 19.9%.
Results: Conspicuous differences were noted in the resistance rates observed in various countries/regions for penicillin (2.4–79.2%), erythromycin (4.0–66.1%), tetracycline (2.4–68.7%), TMP–SMX (3.2–78.0%), chloramphenicol (0–22.2%) and ciprofloxacin (0–7.0%). Countries/regions with consistently highest resistance rates included France, Spain, Hungary, South Africa and Hong Kong–Singapore; consistently lowest resistance rates were noted in Sweden, the Netherlands, Germany, Switzerland and the United Kingdom. The relative percentages of macrolide resistant Spn expressing the efflux and MLSB phenotypes also varied considerably by country/region. The efflux phenotype was more common than the MLSB phenotype in Germany, Switzerland and the United Kingdom; in Hong Kong and Singapore, the percentage of isolates with each phenotype was essentially comparable; in all of the remaining countries/regions surveyed, strains with the MLSB phenotype were conspicuously more common than isolates with the efflux phenotype. Finally, the prevalence of multiply drug resistant strains of Spn also varied markedly by country/region. Generally, MDR Spn were most common in areas characterised by highest overall resistance rates.
Conclusion: Antibiotic resistance with Spn remains a significant problem worldwide, particularly in certain countries.

P1131 Detection of internationally spread multiresistant *Streptococcus pneumoniae* clones in Germany using multi locus sequence typing

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Objectives: Multiresistant *S. pneumoniae* (SP) strains have become a global concern. In global spread of multiresistant SP only a few clonal complexes are widely distributed. By international comparison in Germany resistance in SP is low. The aim of our study was to investigate whether in Germany, despite of low resistance rates, internationally spread multiresistant SP clones occur in isolates recovered from outpatients with RTIs.
Methods: We performed two multicentre studies in Germany in the winter of 2000/2001. MICs were determined using the broth microdilution procedure following NCCLS. Among others erythromycin (ERY), penicillin (PEN), tetracycline (TET) and co-trimoxazole (SXT) were tested. Resistance to ERY and PEN was examined by ermB and mefE duplex-PCR and pbp2b RFLP analysis, respectively. Clonal identity was proven by PFGE. One isolate per clone was analysed by MLST.
Results: Of the 595 SP included, 14.1, 27.6 and 8.1% were resistant to ERY, TET and SXT, respectively. Eighteen per cent were PEN intermediate. With 16 and 17 isolates two multiresistant clones (I and II) dominated in ERY resistance. Clone I, resistant to ERY, TET and SXT and intermediate to PEN, harboured ermB and had unique pbp2b RFLP and PFGE patterns. At three centres in Eastern Germany clone I was found. Clone II was resistant to ERY, TET and SXT, susceptible to PEN, harboured ermB and had a pbp2b RFLP pattern that was typical for PEN susceptible isolates. Its PFGE pattern was unique and was found in two centres in eastern Germany and one centre in southern Germany. The MLST database proved that clones I and II were of international importance. Clone I had sequence type (ST) 135 and was found three times in Spain. Clone II, whose ST was 273, was detected once in Greece, Iceland and Israel, three times in Portugal and twice in Italy. Moreover, clone II, also designated Mediterranian Clone, was related to the Spain-6B-2 clone.
Conclusion: Our results underline the strong impact of a few widely spread multiresistant genetic complexes on resistance in SP at local as well as at the global level. In consideration of this phenomenon one should carefully choose options for the therapy of pneumococcal infections to not select multiresistant SP clones. In Germany, relatively low resistance may be due to the eradication of – also internationally spread – multiresistant clones by high dosed penicillins; however PEN susceptible but ERY resistant strains may be selected by the increasing use of macrolides.

P1132 Trend in antimicrobial susceptibility of nasopharyngeal *Streptococcus pneumoniae* recovered from Italian children: a 7-year study

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Objective: *Streptococcus pneumoniae* (Sp) represents the major cause of bacterial respiratory tract infections in children, with a worldwide increase of its resistance to antimicrobial drugs. We retrospectively determined the trend of antibiotic susceptibility patterns of SP strains recovered from children attending the Gaslini Children's Hospital, Genoa, Italy, in a 7-year period.
Methods: Nasopharyngeal swabs were taken from a total of 2078 children (age 1 month to 14 years) from 1 January 1996 to 10 November 2003. Cultures and species identifications were carried out by conventional methods. Antimicrobial susceptibility to penicillin, ciprofloxacin, cotrimoxazole, chloramphenicol, tetracycline, clindamycin and vancomycin were evaluated by NCCLS standard methods. The erythromycin resistance phenotypes were determined by the double-disk test with erythromycin and clindamycin. Rokitamycin susceptibility was also investigated in 2001–2002.
Results: A total of 469 paediatric patients (23%) were SP colonised, isolation rate was higher in children aged between 2 and 5 years. Nasal swabs were significantly ($P < 0.001$) more efficient than throat swabs in recovering SP (410 vs. 63 strains). The rate of resistance to penicillin decreases, from 23% in 1996–1999 to 19% in 2000–2003, the high level of resistance (MIC >2) decreases from 7.1 to 5.7%; on the contrary, the rate of macrolide-resistance rose from 37% in 1996–1999 to 45% in 2000–2003, with 82% of isolates showing the cMLSB phenotype. The rate of rokitamycin resistance was 10.4% in 2001–2002. Particularly, 50% of penicillin resistance strains had macrolide-resistance with cMLSB phenotype. The resistance to cotrimoxazole was high in all the study periods (14.8%).
Conclusions: Our data show: (i) stable rate of penicillin resistance between 1996 and 2003 in our paediatric patients; (ii) worrisome increase of macrolide resistance rate in our area with a prevalence of cMLSB phenotype strains that could suggest a clonal spread of Sp strains; *iii) a continuous surveillance of antibiotics resistance in Sp in paediatric population is required.

P1133 The International Circumpolar Surveillance System for population-based surveillance of invasive pneumococcal disease, 1999–2002

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Background: The International Circumpolar Surveillance (ICS) Project is a population-based surveillance network for invasive bacterial disease in the US Arctic (AK), Northern Canada (N Can), Greenland (GN), Iceland (IC), Norway (Nor) and Finland (Fin). Among circumpolar countries, the seven-valent conjugate vaccine (pcv7) has been used for routine infant immunisation in AK since 2001 and in selected areas in N Can since 2002.
Methods: We defined a case of invasive pneumococcal disease (IPD) as illness in a surveillance area resident with isolation of *Streptococcus pneumoniae* from a normally sterile site. We analysed data on IPD from AK and N Can (January 1999 to December 2002), and from GN, IC, Nor, Fin (January 2000 to December 2002) to determine: (1) common clinical syndromes, (2) rates of disease by country, (3) serotype distribution and (4) antimicrobial susceptibility patterns.
Results: A total of 5283 cases of laboratory-confirmed IPD were reported from AK (449), N Can (165), GN (26), IC (142), Nor (2643), and Fin (1858). Case–fatality ratios varied from 5.0 to 19.0%. Pneumonia (45%), septicaemia (27%), and meningitis (8%) were the most common clinical presentations. Rates of IPD in aboriginals in AK and N Can were 43 and 45 cases per 100 000 persons, respectively. Rates of IPD in children <2 years of age and persons >2 years of age ranged from 39 to 154 and from 11 to 25 cases per 100 000 persons, respectively. Overall, 67% of isolates from children <2 years of age were serotypes contained in pcv7. In AK, the rate of IPD in children <2 with pcv7 serotypes declined by 80% after routine vaccination; from 137 in 1999–2000 to 28.4 in 2001–2002 ($P < 0.001$); preliminary data from AK for 2003 indicate a rate of 12.3. Rates of non-pcv7 serotypes in AK increased from 26.4 in 1999–2000 to 46.5 in 2001–2002 in children <2 years of age ($P = 0.151$). Overall, 89% of isolates from persons >2 years of age were serotypes contained in the 23-valent polysaccharide vaccine. The proportion of isolates fully resistant to penicillin varied by country from <1% in Fin to 8% in AK.
Conclusions: Rates of IPD are high in aboriginals and children <2 years of age residing in Arctic countries. After introduction of pcv7 in AK, rates of disease in children <2 years of age with pcv7 serotypes rapidly declined; however, increasing rates of non-pcv7 serotypes are concerning and merit further surveillance. Continued surveillance is needed to determine the impact of pcv7 after introduction into other circumpolar countries.

P1134 Trends in beta-lactam resistance in community-acquired lower respiratory tract infection in the UK and Ireland

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Objective: To assess trends in beta-lactam resistance in pathogens associated with community-acquired lower respiratory tract infection in the UK and Ireland.
Methods: 2799 *Streptococcus pneumoniae* and 3736 *Haemophilus influenzae* from lower respiratory specimens were collected from a total of 26 laboratories in the UK and Ireland over the four winters from 1999–2000 to 2002–2003, excluding duplicates within 2 weeks, cystic fibrosis and patients in hospital more than 48 h. Isolates were centrally tested by BSAC agar dilution MIC method and categorised by BSAC breakpoints. Linear trends in proportions were assessed by a chi-squared test.
Results: Only two isolates (of *S. pneumoniae*) were resistant to cefotaxime, both in Ireland, one in each of the first 2 years. Rates of resistance to amoxicillin (AMX), cefuroxime (CXM), ampicillin (AMP) and amoxicillin/clavulanate (AMC), non-susceptibility to penicillin (PEN), and beta-lactamase (B-LAC) production are shown in the table. The only significant trend in the UK was a reduction in CXM resistance in *H. influenzae*. In Ireland, starting from a higher baseline, there were significant trends towards reduced resistance in *S. pneumoniae* for PEN, AMX and CXM and in *H. influenzae* for B-LAC and AMP. However, cautious interpretation is required as the sample includes few centres in Ireland.

	1999–2000	2000–2001	2001–2002	2002–2003	*P*
S. pneumoniae, UK, *n*	595	608	631	683	
PEN %I+R	7.4	7.1	4.6	6.4	0.24
AMX %R	0.3	1.2	0.5	1.0	0.37
CXM %R	4.8	3.6	3.3	4.1	0.48
S. pneumoniae, Ireland, *n*	66	59	68	89	
PEN %I+R	41	42	37	25	0.02
AMX %R	9	3	4	0	0.01
CXM %R	38	34	25	21	0.01
H. Influenzae, UK, *n*	863	861	822	830	
B-LAC % positive	14.0	15.4	16.1	16.4	0.16
AMP %R	14.5	15.6	16.7	16.1	0.27
AMC %R	7.6	3.8	5.2	5.2	0.08
CXM %R	18.8	16.7	17.4	13.4	0.01
H. Influenzae, Ireland, *n*	73	97	94	96	
B-LAC % positive	22	19	12	7	<0.01
AMP %R	23	20	13	9	0.01
AMC %R	10	5	5	4	0.18
CXM %R	22	7	16	10	0.18

R resistant (MIC $\geq$ 2 mg/L). I intermediate (MIC 0.12–1 mg/L).
P value for test of linear trend.

Conclusion: Beta-lactam resistance in community-acquired lower respiratory *S. pneumoniae* and *H. influenzae* is no longer rising in the UK and may be falling in Ireland.

P1135 Susceptibility of *Streptococcus pneumoniae* respiratory isolates from adults in Spain (2001–2002)

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Objectives: Surveillance of antibiotic resistance of *S. pneumoniae* is a clear need mainly in countries with high resistance rates. Susceptibility results of pneumococci isolated from adults to commonly prescribed oral antibiotics in Spain are presented. The impact of the new oral pharmacokinetically enhanced coamoxiclav 2000/125 mg formulation is also shown.
Methods: A prospective, multicentre (25 hospitals) antimicrobial survey was carried out between November 2001 and October 2002. A total of 2348 consecutive *S. pneumoniae* isolates from patients with community-acquired respiratory tract infections were collected and sent to a central laboratory for further processing. Susceptibility testing was performed by a semiautomated microdilution method following NCCLS M100-S12 guidelines and breakpoints against antibiotics commonly used, except for ciprofloxacin (resistant if MIC greater than or equal to 4 mg/L). New enhanced coamoxiclav 2000/125 mg allows for shifting to the right one dilution the NCCLS breakpoints for coamoxiclav.
Results: MIC50/MIC90 (mg/L), and percentage of fully resistance are given for each antibiotic: penicillin (less than or equal to 0.01/2;

20.3%); coamoxiclav (0.03/2; 4.6%); cefaclor (1/greater than or equal to 16; 35.5%); cefuroxime–axetil (less than or equal to 0.12/8; 25.4%); erythromycin (0.06/greater than or equal to 64; 32.4%); ciprofloxacin (1/2; 5.1%). Clarithromycin and azithromycin behaved exactly like erythromycin. Augmentin Plus reduces the high resistance to former coamoxiclav until only 0.4% (a 91.3% reduction). By shifting the NCCLS breakpoints one dilution, reduction of co-resistance with coamoxiclav 2000/125 is shown in the Table.

	Full resistance (%) to:	
Among isolates resistant to:	Current coamoxiclav NCCLS breakpoints	New PK/PD enhanced coamoxiclav breakpoints
Penicillin	22.7%	1.9%
Cefaclor	13.0%	1.1%
Cefuroxime-axetil	17.8%	1.5%
Erythromycin	6.3%	0.9%
Ciprofloxacin	10.0%	0.8%

Conclusions: (1) While erythromycin resistance remains above 30%, penicillin resistance seems to be decreasing (20.3%) with respect to previous surveillances. (2) Erythromycin, clarithromycin, azithromycin and cefaclor displayed the worst intrinsic activity and the highest resistance rates. (3) Coamoxiclav and penicillin were the most active drug in terms of MIC50 and MIC90. Coamoxiclav presented the lowest prevalence of resistance. (4) The potency of the new oral pharmacokinetically enhanced coamoxiclav 2000/125 mg formulation (with only a 0.4% of resistance) means additionally the almost virtual disappearance of the problem of co-resistance in *S. pneumoniae*.

P1136 Serotypes and antimicrobial resistance of *Streptococcus pneumoniae*: evolution (1989–2003) in Asturias, Spain

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Objectives: To assess the prevention and the treatment strategies in pneumococcal disease, we have analysed the serogroups/seotypes (SGTs) and the antibiotic resistance in our geographical area during 1989–2003.
Methods: Invasive strains, from adult patients, were studied. The serotyping was performed in the national reference centre. The oxacillin disk and the broth microdilution method (NCCLS M100-S13) were used. The distribution was determined during three periods: I 1989–1993, II 1994–1999, III 2000–2003. The chi-square test was used.
Results: 510 invasive isolates were identified (421 blood, 49 CSF, 40 others). The most frequent SGTs (%) were: 3 (19.6), 14 (10.5), 19 (7.8), 4 (7.8), 9 (7.6), 8 (6.6), 6 (6.4) and 23(4.5). The distribution (%) in the periods I ($n = 192$), II ($n = 139$), III ($n = 179$) was: SGT 3: 21.8, 18.7, 17.8; SGT 14: 8.8, 8.6, 13.9; SGT 19: 6.2, 7.1, 10.0; SGT 4: 5.2, 3.5, 13.9; SGT 9: 9.8, 10.7, 2.7; SGT 8: 4.6, 8.6, 7.2; SGT 6: 7.8, 6.4, 5.0 and SGT 23: 6.7, 4.3, 2.2. Relevant trends between periods I, III were: SGT 4, 5.2 vs. 13.9% ($P = 0.003$), SGT 9, 9.8 vs. 2.7% ($P = 0.005$) and SGT 23, 6.7 vs. 2.2% ($P = 0.036$). The coverage of the 23-valent vaccine for all SGTs was 96.2% and 95.6, 94.1, 98.3% in periods I, II, III, respectively. The coverage for SGTs in the elderly (more than 64 years) was 97.2%. The resistance to penicillin was 30.3% (22.3% intermediate, 8.0% resistant); the evolution was: 31.2, 33.8 and 26.8% ($P = 0.347$). The trend in erythromycin resistance was 5.7, 16.5 and 23.4%; the progressive increase was very significant among periods I and III ($P < 0.0001$). The prevalence of resistance (%) to tetracycline and chloramphenicol was 35.4, 25.1, 21.7 and 23.9, 16.5, 7.2, respectively.
Conclusions: There were no significant changes in the SGTs' distribution. The prevention with the 23-valent vaccine can be recommended in this population. The resistance to penicillin remains stable, by contrast the resistance to macrolide has increased dramatically. Continuous monitoring is mandatory.

P1137 Investigation of genetic relatedness among penicillin-nonsusceptible pneumococci isolated in Turkey

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Objective: To analyse the clonal relationship among penicillin-nonsusceptible *Streptococcus pneumoniae* isolates recovered at different medical centres in Turkey by different methods.
Methods: The isolates consisted of 90 penicillin-nonsusceptible (10 high level resistant (PenR) and 80 low level resistant (PenI)) and 20 consecutive penicillin-susceptible *S. pneumoniae* which had been isolated at seven different centres between November 1999 and January 2001. Clonal relationship between the isolates were investigated by the analysis of restriction endonuclease patterns of pbp (1a, 2b, 2x) genes (PBP-REA) and by BOX-PCR using the BOX-A1R primer. Antibiotic susceptibility patterns and capsular serotypes were also determined.
Results: 12, 8 and 15 different PBP-REA patterns were detected by the analysis of pbp 1a, 2b and 2x, respectively. The combination of these revealed a total of 55 REA patterns among the isolates. The most common PBP-REA pattern (pattern 1/2/1) observed in penicillin-nonsusceptible strains, was not detected among the susceptible isolates. Likewise, the most common pattern of the susceptible strains (pattern 2/4/5) was specific to only this group. When BOX-PCR was used, 8, 62 and 18 different patterns were identified among PenR, PenI and PenS isolates, respectively.
Conclusions: (i) PBP-REA patterns of the isolates were more limited when compared with BOX-PCR results which can be explained by different specificities of the techniques, namely the whole chromosome for BOX-PCR and specific pbp genes for REA. (ii) Although discrepancies exist between different methods, a significant clonal relationship and spread was not detected among penicillin-nonsusceptible pneumococci isolated in Turkey.

P1138 Selection of single step parC mutations among levofloxacin susceptible *S. pneumoniae* in Canada

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Objectives: The fluoroquinolones were first introduced into routine clinical practice for the management of community acquired RTIs in Canada during the late 1990s. Despite extensive penetration into the community, fluoroquinolone resistance in *S. pneumoniae* remains low. However, in this study, we document the selection of first step parC mutations among levofloxacin susceptible strains.
Methods: *S. pneumoniae* were collected from both respiratory tract specimens and sterile sites from the Maritime region of Canada as part of a regional surveillance programme. Isolates collected in 1999, 2002, and 2003 with MICs to levofloxacin of 1 and 2 μg/mL were analysed. MICs to levofloxacin and moxifloxacin were determined using a microbroth dilution method. The QRDR regions of gyrA and parC were amplified by PCR and their DNA sequence determined. PFGE was performed using SmaI and standard methods.
Results: 50 isolates with an MIC to levofloxacin of 1 μg/mL from each year (150 strains), and 15, 28, and 34 isolates with an MIC to levofloxacin of 2 μg/mL, respectively, were selected for study. The MIC90s to levofloxacin and moxifloxacin were 1 and 0.25 μg/mL, respectively. In 1999, <2 and 6% of isolates with MICs of 1 and 2 μg/ml (levofloxacin), respectively, harboured parC mutations. In 2002, 6 and 14% of isolates with MICs of 1 and 2 μg/mL, respectively, harboured parC mutations and in 2003, 6 and 48% of isolates with MICs of 1 and 2 μg/mL, respectively, harboured parC mutations. No evidence of clonal dissemination was

observed among the isolates. Only one isolate was found to contain a single step gyrA mutation.
Conclusions: Fluoroquinolone resistance among *S. pneumoniae* in the Maritime region of Canada remains low, however, a significant increase in isolates containing a parC mutation has been observed in the last 12 months. The selection of parC mutations is may be related to the use of levofloxacin to treat *S. pneumoniae* infections. Conversely, use of the 8-methoxyfluoroquinolones such as moxifloxacin, that preferentially select for DNA gyrase mutations, does not appear to be contributory as evidenced by the isolation of only one strain with a single gyrA mutation.

P1139 Evolution of macrolide resistance in *Streptococcus pneumoniae* in Italy

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Objectives: In the past years, resistance of *Streptococcus pneumoniae* to erythromycin and the other macrolides has increased in many parts of the world, including Italy. The most important mechanisms of macrolide resistance involve the erm(B) gene, conferring resistance to macrolides, lincosamides and streptogramin B, and the mef(A) gene, encoding a drug efflux pump. Our aim was the study of the evolution of erythromycin resistance in *S. pneumoniae* invasive isolates in Italy.
Methods: 934 isolates from blood or CSF collected from patients in several Italian hospitals were studied: 503 from the period 1997/2000 and 431 from the period 2001/2003. Susceptibility tests to antimicrobial agents were determined by Etest and Sensititre panels. A PCR assay was performed to detect the presence of erythromycin resistance determinants. By this assay the erm(B) gene and the two subclasses of the mef(A) gene, mef(A) and mef(E), could be recognised. Capsular serotyping was performed in all strains using the Pneumotest panel.
Results: Out of 503 isolates from the period 1997/2000, 145 (29%) were resistant to erythromycin; out of these, 106 (73%) carried erm(B) and 39 (27%) carried mef(A); out of these, 6 (15%) carried subclass mef(E). Out of 431 isolates from the period 2001/2003, 150 (35%) were resistant to erythromycin; out of these 89 (59%) possessed erm(B), 57 (38%) possessed class mef(A), 3 isolates possessed both genes and one neither of these genes. Out of 57 mef(A) strains 9 (16%) carried subclass mef(E). In both periods the most frequent serotypes of erythromycin resistant strains were 14, 19F and 6B. All subclass mef(A) strains, except two strains isolated in 2001/2003, belonged to serotype 14, while subclass mef(E) strains belonged to different serotypes (14, 9V, 11A, 6B).
Conclusions: In Italy, erythromycin resistance in *S. pneumoniae* has been increasing steadily. The majority of the erythromycin resistant isolates carry erm(B). However, the increase is mainly due to the increase in the number of isolates carrying mef(A). Moreover, in the more recent period we observed the appearance of strains carrying both genes. The distribution of resistance genes and serotypes suggest that the increase of macrolide resistance is due to a combination of clonal expansion and horizontal transmission.

P1140 Evolution of levofloxacin activity against *Streptococcus pneumoniae* in France (1999–2002)

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Levofloxacin (LVX) was the first antipneumococcal fluoroquinolone (FQ) available by parenteral and oral route introduced for therapeutic use in France, at the end of 1999. Three follow-up surveys of its *in vitro* activity against *Streptococcus pneumoniae* (SP) have been initiated since this time, in France.
Methods: The first survey (S1) was conducted during the respiratory season 1999–2000 and was representative of the epidemiology before the clinical use of LVX. The second (S2) was done during the respiratory season 2000–2001, and the third (S3) during the year 2002 corresponding to the status of LVX activity after 1 and 2 years of use, respectively. Thirty-five, 30 and 42 French metropolitan hospital laboratories participated in the S1, S2 and S3 surveys, respectively. In the three surveys, SP strains were isolated from respiratory tract infections. LVX *in vitro* activity was determined by disc diffusion in S1 (with a good correlation with MICs by agar dilution against a sample of strains) and by MIC determination in S2 and S3. All strains were isolated from adult patients during S2 and S3 and 79% during S1. Quality control was performed with SP ATCC 49619. Susceptibility rates were calculated according to the recommendations of the Comité de l'Antibiogramme de la Société Française de Microbiologie.
Results: 477, 675 and 965 strains were, respectively, isolated during the S1, S2 and S3 surveys. The rates of strains susceptible (S), intermediate (I) and resistant (R) to LVX were the following: S1: 97.3% S, 1% I and 1.7% R; S2: 99% S, 0.1% I and 0.9% R; S3: 98.6% S, 0.1% I and 1.2% R.
Conclusions: LVX demonstrates a very good activity against SP with less than 2% of resistant strains. LVX resistant strains were pre-existing to the introduction of antipneumococcal FQs in France and did not increased after introduction of LVX on the French market.

P1141 Trends of ceftriaxone resistance in Canadian strains of *Streptococcus pneumoniae*

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Objectives: In January 2002, NCCLS published new susceptibility interpretive criteria for ceftriaxone for non-meningeal isolates of *S. pneumoniae* (SP). Previous susceptibility interpretive breakpoints were $\leq 0.5/1/\geq 2$ μg/ml for S/I/R, respectively. The new interpretive criteria for susceptibility in non-meningitis are $\leq 1/2/\geq 4$ μg/ml for S/I/R, respectively. The criteria for meningeal isolates remain unchanged. We examine the trends of ceftriaxone resistance under the new interpretive guideline for both groups of isolates.
Methods: In 1988 and from 1993 to present, 19 323 SP isolates from 192 labs were submitted for susceptibility testing according to NCCLS protocols. We analysed the activity of ceftriaxone against 19109 strains of SP isolated from CSF and non-CSF specimens applying the old interpretive breakpoint prior to January 2002 vs. the new breakpoint.
Results: Of the 19 109 isolates, 269 were from CSF, 18 840 were from non-CSF specimens (6532 from respiratory specimens and 5218 from other sites). The percentage of resistance (R) and intermediate (I) by year is shown in the following table.

	Penicillin		Ceftriaxone							
	All Source		CSF			Non-CSF				
							Old Guideline		New Guideline	
Year	I	R	N	I	R	N	I	R	I	R
2003	7.5	5.8	10	20.0	0	944	4.6	2.0	1.7	0.3
2002	8.5	6.7	32	6.3	0	2507	6.2	1.5	1.4	0.2
2001	7.7	6.8	28	10.7	3.6	2230	4.9	2.4	2.3	0.1
2000	6.7	5.9	44	6.8	2.3	2226	4.9	2.0	1.9	0.1
1999	7.6	5.9	20	10.0	5.0	2131	4.8	1.5	1.2	0.3
1998	9.2	5.6	17	11.8	0	1444	4.0	2.4	2.3	0.1
1997	6.9	6.5	19	5.3	0	1525	5.5	1.3	1.2	0.1
1996	8.0	4.1	32	0	3.1	1153	4.0	0.6	0.5	0.1
1995	6.6	2.2	30	0	0	1504	1.7	0.1	0.1	0
1994	6.8	1.3	29	0	0	2811	0.8	0.2	0.1	0
1993	4.7	0.9	2	0	0	210	0.5	0	0	0
1988	2.5	0	6	0	0	155	0	0	0	0

Penicillin susceptibility is included for comparison.
Conclusions: Using the new interpretive criteria, we found that only 0.14% of all SP isolates were resistant to ceftriaxone. Resistance in CSF isolates (1.5%) is higher than non-CSF isolates (0.12%). Because the sample size of CSF isolates is very small, further study is required to determine the significance. Ceftriaxone resistance in pneumococci rapidly increased until 1998 but has since remained stable. This holds true for both groups of isolates interpreted under both the old and the new criteria. Resistance to ceftriaxone is low compared with other antibiotics including penicillin.

P1142 Isolation and resistance patterns of *S. pneumoniae* in a 6-year period (1997–2003)

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Objectives: The aim of the present study was to investigate the resistance patterns of *S. pneumoniae* isolates consecutively collected in the Microbiology Department of 'Sotiria' Chest Diseases Hospital of Athens, from 1993 to 2003.
Methods: In total, 282 clinical isolates were investigated. The susceptibility of *S. pneumoniae* was tested against penicillin, cefotaxime, erythromycin, tetracyclin, trimethoprim/sulfamethoxazole and ciprofloxacin. The standard disc diffusion method was performed for all isolates, as described in the guidelines of the National Committee for Clinical Laboratory Standards. The susceptibility for penicillin was detected with oxacillin 1 μg discs and the MICs by the Etest method (AB Biodisk, Sweden). Concerning the site of infection, 210/282 (74.5%) isolates were isolated from sputum, while 72/282 (25.5%) from invasive infections, mostly bacteraemia (>50%).
Results: From the total of 282 isolates, 52 (18.4%) were non-susceptible to penicillin. The MIC detection revealed that 37/52 (71.1%) were intermediate and 15/52 (28.9%) fully resistant to penicillin (MICs 0.12–1.0 and >2, respectively), while 1/52 (1.9%) was resistant to cefotaxime (MIC>2). Regarding the clinical source, 41/52 (78.9%) of the PNSP strains were isolated from sputum and 11/52 (21.2%) from invasive infections. From the total of 282 isolates, 86 (30.2%) were resistant to erythromycin. Regarding the PNSP isolates, 34/52(65.3%) were erythromycin resistant, while 52/230 (22.6%) of the penicillin sensitive isolates were erythromycin resistant. In addition, 35/52 (67.3%) PNSP isolates were multi-resistant, while 52/230 (22.6%) penicillin susceptible isolates were multi-resistant.
Conclusion: Referring to a previous study of ours, for the period 1992–1993, it is demonstrated that both the PNSPs as well as the erythromycin resistant isolates show an increasing incidence of 4.5 and 10.2%, respectively.

P1143 Macrolide and quinolone resistance mechanisms in *Streptococcus pneumoniae* population from children in day-care centres and orphanages in Asian Russia

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Objective: Data on pneumococcal carrier rates in Asian Russia are sparse. *S. pneumoniae* isolates (SPIs) were collected from children <5 years in five cities of Asian Russia. The aim was to determine distribution of macrolide and fluoroquinolone resistance mechanisms and clonal relatedness in the *S. pneumoniae* population among paediatric carriers.
Methods: A total of 866 SPIs isolated from nasopharyngeal swabs of carriers in Asian Russia (Habarovsk, Vladivostok, Tyumen, Novosibirsk and Yakutsk) were studied by microdilution MIC (NCCLS). Macrolide resistance genes erm(B), and mef were tested by PCR. Genes encoding L4, L22 and 23S rRNA in erm(B)/mef negative strains and topoisomerase IV and DNA gyrase genes in SPIs with raised ciprofloxacin MICs (all 4 μg/mL) were sequenced. PFGE analysis was done.
Results: Of 866 SPIs 55 (6.3%) had raised MICs: 40 to macrolides and 15 to quinolones. Of 40 macrolide resistant SPIs four (10%) were mef positive and 16 (40%) had erm(B). In 20 erm(B)/mef negative SPIs seven had S20N change in L4 protein and three strains possessed additional 69GTG71 to TPS substitution in L4; 16 (including four strains with alteration in L4) had a mutation in L22 and 6 SPIs had additional nucleotide substitution in 23S rRNA. The resistance mechanism for one strain is unknown. Of 15 SPIs with raised cipro MICs, 14 (93.3%) had changes in ParE: I461V or A, V460A, A466D, A488D; three strains had additional mutation in ParC (K137N) and 1 strain had a substitution in GyrA (A149G). Macrolide resistant SPIs with erm(B) were associated with three PFGE types ($n = 11$) and five erm(B) strains as well as all four mef strains, were unique. All strains with 69GTG71 to TPS substitution in L4 shared one PFGE type (clones), but different serotype (23 or 19). SPIs with raised cipro MICs were mostly unique. Most common serotypes among resistant SPIs ($n = 72$) were 23 ($n = 26$), 6 ($n = 20$) and 19 ($n = 17$). Remaining nine strains had serotypes: 3, 9V, 12, 14 and 18.
Conclusions: Macrolide resistance mechanism in strains from nasopharyngeal carriers in Asian Russia resulted mainly from presence of erm(B) but also mef gene and mutations in ribosomal proteins. Surprisingly 93.3% strains with raised cipro MICs had one, double or triple change in ParE.

P1144 Serotype and antimicrobial susceptibility of *Streptococcus pneumoniae* isolated in non-invasive disease in Portugal

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Lisbon, P

Objectives: *Streptococcus pneumoniae* is a leading cause of pneumonia and other respiratory tract infections. The aim of this study was to evaluate the serotype-antimicrobial susceptibility of non-invasive pneumococci isolated in Portugal.
Methods: We studied 445 non-invasive *S. pneumoniae*, with low or high resistance to penicillin (MIC$\geq$0.1 mg/L), isolated from different specimens in 16 Portuguese hospitals, and collected in the Antibiotic Resistance Unit in National Institute of Health, between 1994 and 2000. MICs (mg/L) to eight antibiotics were determined by agar dilution method (NCCLS). Serotype was performed by Dot-Blot and Quellung reaction.
Results: 42% of penicillin resistant *S. pneumoniae* showed resistance against cefotaxime (Ctx) and 35% against ceftriaxone (Ctr); 45, 23, 18, 31, and 2% were resistant against tetracycline (Tet), erythromycin (Ery), chloramphenicol (Cm), clindamycin (Cli) and ofloxacin, respectively. Serotypes 23F, 9V, 14, 15A, 19F, 6B, 6A (in descending order) represented 90% of the isolates causing non-invasive pneumococcal disease. Multidrug resistance reached 43% of penicillin resistant strains. The prevalent multidrug resistant phenotype was Pen plus Ctx plus Ctr plus Tet plus Cm (mainly from serotype 23F, 96%), followed by the phenotype Pen plus Ctx plus Ctr plus Ery plus Cli (mainly from serotype 15A, 33%). Overall were detected 25 different multidrugresistant phenotypes. Penicillin resistant strains from serotype 23F diminished from 44 to 23% between 1994 and 2000, in opposite to serotype 14 which increased from 6 to 33%, in the same period.
Conclusions: In this study we showed that penicillin resistance and multidrug resistance was mostly associated with non-invasive *S. pneumoniae* strains of serotype 23F. Serotypes found among the sample (15A, 5% and 6A, 3%) are not included in the pneumococcal 7-valent conjugate vaccine. Our work highlights the importance of monitoring the serotype and antimicrobial susceptibility of isolates from patients with non-invasive pneumococcal disease in Portugal.

P1145 Antimicrobial susceptibility and serotype distribution of invasive *Streptococcus pneumoniae* isolated in Portuguese children under 5 years old (1999–2002)

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Objectives: To establish a relationship between serotypes and antimicrobial resistant patterns in Invasive Pneumococcal Disease (IPD) in children under 5 years old in Portugal.
Methods: 151 consecutive isolates were collected in the Antibiotic Resistance Unit in National Institute of Heath, from blood, CSF and pleural liquid, between 1999 and 2002, in the scope of a multicentre study with the participation of 16 hospitals; 55% of strains were isolated in males and 45% in females. MICs to 10 antibiotics were determined by agar dilution method (NCCLS). Serotype was performed by Dot-Blot and Quellung reaction with antiserum from Statens Serum Institute.
Results: MICs90 (mg/L) ranged as follows: 0.5–1.6 to penicillin, 0.25–1 to cefotaxime, 0.5–1 to ceftriaxone, 2–64 to tetracycline, 8–32 to erythromycin, 32–64 to clindamycin, 4 to chloramphenicol, 2 to ofloxacin and 1–2 to ciprofloxacin. Serotypes 14, 1, 23F, 6B, 7F, 19F, 3, 9V (in descending order) represented 80% of the isolates causing IPD in children. Serotype 14 was the most important in children under 2 years old (34%) and serotype 19F was the most important in the age group 3–5 years old (21%). Serotypes 14, 1, 6B and 7F were more frequent in blood then in CSF and serotypes 23F, 19F and 6A in CSF than in blood. The main serotypes of penicillin resistant strains were: 14, 23F, 9V, 6B, 19A and 19F (in descending order). Serotypes 14, 6B, 19F, 33F and 15C (in descending order) were prevalent among erythromycin resistant strains. Tetracycline resistant isolates were mainly from serotypes 6B, 19F, 14 and 33F (in descending order).
Conclusions: Our results suggest that the pneumococcal 7-valent conjugate vaccine should protect against 95, 86, and 81% of penicillin, tetracycline, erythromycin resistant strains, respectively, and against 100% of cefotaxime and ceftriaxone resistant strains. This vaccine cover 61% of IPD in the paediatric Portuguese population under 5 years old and the vaccine serotypes plus vaccine-related serotypes cover 69%. Monitoring of serotypes and antimicrobial susceptibility is of high concern in Portugal in terms of Public Health.

P1146 Results of multicentre study of antimicrobial resistance of nasopharyngeal *Streptococcus pneumoniae* in children from day-care centres and orphanages in Asian Russia (SPARS-ASIA study)

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Objectives: There are no prospective data on resistance to antimicrobials amongst nasopharyngeal pneumococcal carriers in Asian Russia. A single group of clinicians and microbiologists performed sampling of children during 2001–2002, followed by isolation and susceptibility testing of strains using a unified methodology.
Methods: Nasopharyngeal swabs were collected from 1669 children <5 years from 40 day-care centres and orphanages in eight cities of Asian Russia (Anadyr, Irkutsk, Khabarovsk, Khanty-Mansiysk, Novosibirsk, Tyumen, Vladivostok, Yakutsk) with immediate plating on to 5% Columbia blood agar with 5 mg/L gentamicin. Susceptibility testing to penicillin G (PEN), amoxicillin (AMO), amoxicillin/clavulanate (AMC), cefotaxime (CTX), erythromycin A (ERY), azithromycin (AZI), clarithromycin (CLA), clindamycin (CLI), telithromycin (TEL), ciprofloxacin (CIP), levofloxacin (LEV), gemifloxacin (GEM), tetracycline (TET) and co-trimoxazole (SXT) was performed by NCCLS microdilution. Breakpoints were those of NCCLS except for TEL (equal or less than 0.5; 1–2; >2 mg/L), CIP (equal or less than 2; 4; more or equal to 8 mg/L), GEM (equal or less than 0.12; 0.25; more or equal to 0.5 mg/L).
Results: A total of 912 *S. pneumoniae* were isolated, with carriage rate varying from 11.1% to 86.7% between institutions. Susceptibility testing results are presented in the Table.

Antimicrobial	%I (*n*)	%R (*n*)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	MIC range (mg/L)
PEN	15.4 (140)	1.0 (9)	0.06	0.25	0.008–4
AMO	0.1 (1)	0	0.03	0.125	0.03–4
AMC	0.1 (1)	0	0.03	0.125	0.03–4
CTX	0.3 (3)	0	0.016	0.125	0.008–2
ERY	0.2 (2)	5.7 (52)	0.03	0.06	0.016–128
AZI	0.6 (5)	5.6 (51)	0.06	0.125	0.03–128
CLA	0.6 (5)	5.7 (52)	0.03	0.06	0.016–128
CLI	0.2 (2)	2.5 (23)	0.03	0.06	0.016–128
TEL	0	0	0.016	0.03	0.002–0.5
CIP	2.1 (19)	0	1	2	0.125–4
LEV	0	0	0.5	1	0.125–2
GEM	0	0	0.016	0.06	0.016-0.125
TET	4.9 (45)	50.6 (461)	8	32	0.25–64
SXT	42.7 (389)	25.4 (232)	1	8	0.06–16

Conclusions: (i) Pen I and Pen R were found in 15.4 and 1.0% of strains, respectively. All but one strain were AMO-S and AMC-S; (ii) Macrolide non-susceptibility occurred in 5.9–6.3% of strains and all were S to TEL with low rates of increased quinolone MICs; (iii) High rates of R to TET and SXT were detected.

P1147 Antimicrobial susceptibility of *Streptococcus pneumoniae* isolated from adults with acute sinusitis in three Russian centres

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Objectives. The purpose of this study was to determine the susceptibility of the *S. pneumoniae* causing acute sinusitis (AS) in adults.
Methods. A total of 142 *S. pneumoniae* isolated from aspirates obtained via maxillary sinus punctures in Smolensk (S), Moscow (M) and St. Petersburg (SP) were studied. Susceptibility to penicillin G, amoxicillin, amoxicillin/clavulanate, cefotaxime, cefepime, erythromycin, azithromycin, clarithromycin, clindamycin, tetracycline, levofloxacin, moxifloxacin, chloramphenicol and co-trimoxazole was determined by broth microdilution according to NCCLS (2003) guidelines.
Results. The most active antimicrobials were amoxicillin, amoxicillin/clavulanate, cefotaxime, cefepime, levofloxacin and moxifloxacin to which no resistance was found. Intermediate resistance to penicillin G was 4.2% (6.5, 4.3 and 1.8% in S, M and SP, respectively). Proportion of non-susceptible strains to macrolides, chloramphenicol and clindamycin was 1.4% (S, 0%; M, 4.3%; SP, 1.8%), 4.9% (S, 3.2%; M, 4.3%; SP, 7.0%) and 0.7% (S, 0%; M, 0%; SP, 1.8%), respectively. The highest percentage of non-susceptible isolates was found to tetracycline and co-trimoxazole – 28.2% (S, 30.6%; M, 30.4%; SP, 24.6%) and 41.6% (S, 35.4%; M, 30.4; SP, 52.7%), respectively.
Conclusion. *S. pneumoniae* retained their susceptibility to aminopenicillins, III–IV generation cephalosporins and respiratory fluoroquinolones. The highest non-susceptibility was found to tetracycline and co-trimoxazole, substantially compromising possibility of their usage for empiric therapy of AS.

P1148 Distribution of macrolide resistance mechanisms in *Streptococcus pneumoniae*: 3-year update from the PROTEKT study

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Objectives: PROTEKT — a global, longitudinal study of the antimicrobial susceptibility of bacterial community-acquired respiratory tract pathogens — has completed its third year (Year 1: 1999–2000; Year 2: 2000–2001; Year 3: 2001–2002). This is an update on the distribution pattern of macrolide resistance mechanisms among *S. pneumoniae*.
Methods: Erythromycin nonsusceptible (intermediate and resistant, MIC $\geq$0.5 mg/L; ERY-I/R) *S. pneumoniae* isolates were tested centrally for the presence of macrolide resistance genes [erm(B), mef(A) and erm(A) subclass erm(TR)] using PCR.
Results: Genotyping data are available for 4818 ERY-I/R S. pneumoniae isolates (Year 1: 1084; Year 2: 1397; Year 3: 2337) collected from 32 countries. As in previous years, erm(B) alone and mef(A) alone were the most prevalent resistance mechanisms worldwide in Year 3, accounting for 57.7 and 30.6% of ERY-I/R isolates tested, respectively. Over the 3 years, the erm(B) genotype predominated in many European countries, while mef(A) was particularly prevalent in the UK, USA, Argentina, Peru and Austria. Overall, 10.1% of Year 3 ERY-I/R isolates contained both erm(B) and mef(A), with the highest prevalence found in South Africa and South Korea (occurring in 41.6% [131/315] and 37.0% [114/308] of all ERY-I/R isolates, respectively). The overall proportion of isolates with ribosomal mutations remained low worldwide (1.7, 1.5 and 1.4% in Years 1, 2 and 3, respectively), but accounted for 9.2% (20/217) of all ERY-I/R isolates from Canada. Approximately half of all ERY-I/R isolates were resistant to penicillin, with co-resistance most common in isolates with dual macrolide resistance mechanisms (89.7%). Susceptibility of macrolide-resistant isolates to the ketolide antibacterial telithromycin remained unchanged over the 3 years of investigation. Overall, telithromycin mode MIC and MIC90 values were, respectively, 0.03 and 0.25 mg/L against erm(B) strains, 0.06 and 0.25 mg/L against mef(A) strains and 0.5 and 0.5 mg/L against isolates with both erm(B) and mef(A).
Conclusions: Mechanisms of macrolide resistance continue to vary worldwide; however, telithromycin retains high activity against macrolide-resistant *S. pneumoniae*, irrespective of resistance mechanism.

P1149 Susceptibility of *Streptococcus pneumoniae* to penicillin, azithromycin and telithromycin (PROTEKT 1999–2002)

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Objectives: PROTEKT is a global, longitudinal surveillance programme established in 1999 to study the antimicrobial susceptibility of common bacterial pathogens associated with community-acquired respiratory tract infections (RTIs). This analysis was undertaken to determine the susceptibility of *Streptococcus pneumoniae* to penicillin, azithromycin and telithromycin and to assess the prevalence of co-resistance between these antibiotics.
Methods: *S. pneumoniae* isolates have been collected from 32 countries as part of the PROTEKT programme over three consecutive respiratory seasons (Year 1: 1999–2000; Year 2: 2000–2001; Year 3: 2001–2002). MICs for penicillin, azithromycin and telithromycin were determined centrally by NCCLS broth microdilution methods and interpreted using NCCLS breakpoints.
Results: Data are available for a total of 14 011 isolates of *S. pneumoniae* collected between 1999 and 2002. Mode MIC, MIC90 and MIC range were, respectively, 0.015, 2 and $\leq$0.008 to >4 mg/L for penicillin and 0.12, >64 and $\leq$0.03 to >64 mg/L for azithromycin. In all, 13.6% of isolates were found to have intermediate susceptibility to penicillin (PEN-I; MIC 0.12–1 mg/L) and 21.6% were penicillin resistant (PEN-R; MIC $\geq$2 mg/L). Similarly, 33.9% were resistant to azithromycin (AZI-R; MIC $\geq$2 mg/L), with 22.9% of isolates having an azithromycin MIC of >64 mg/L. A total of 55.3% of PEN-I isolates and 73.0% of PEN-R isolates were found to be co-resistant to azithromycin, with 22.2% of AZI-R isolates having intermediate susceptibility to penicillin and 46.5% being fully resistant. Telithromycin was more potent than the other two antibacterials tested, with a mode MIC of 0.008 mg/L, an MIC90 of 0.12 mg/L and an MIC range of $\leq$0.002–8 mg/L. Telithromycin retained high activity against PEN-R and AZI-R isolates, with 99.7% of PEN-R isolates and 99.8% of AZI-R isolates susceptible to telithromycin (MIC $\leq$1 mg/L) using tentative breakpoints as approved by the NCCLS Antimicrobial Susceptibility Testing Subcommittee, January 2003.
Conclusions: Over one-third of all *S. pneumoniae* isolates collected between 1999 and 2002 were found to have reduced susceptibility to penicillin or azithromycin, with co-resistance to these antibacterials apparent in the majority of cases. In contrast, telithromycin was found to have potent antibacterial activity against this major community-acquired RTI pathogen, with $\geq$99.7% of PEN-R and AZI-R strains retaining susceptibility to telithromycin.

P1150 Serotype distribution and antimicrobial resistance of *Streptococcus pneumoniae* in Austria, 1996–2002: Implications for vaccination strategies

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Streptococcus pneumoniae is a major cause of morbidity and mortality worldwide and the most common cause of severe diseases like meningitis or community-acquired pneumonia. In this study a total of 2367 strains of *S. pneumoniae* were collected in an Austrian-wide surveillance system from 1996 to 2002. Isolates were tested on their susceptibility to penicillin and clarithromycin and serotyping was performed by the capsular swelling method. Overall, a rise in penicillin resistance from 4.9% in 1996 to 10.0% in 2002 (including both intermediate-resistant and resistant strains) could be observed. Also, a distinct rise in macrolide resistance was recorded in this period. The overall distribution of serotypes remained relatively stable with serotypes 23, 19, 6 and 14 being the most frequent ones. While in 1996 penicillin resistance was predominantly associated with serotype 23F, in 1998 serotype 9 and in 2002 serotype 14, was most frequently found in these resistant strains. Coverage rates for currently available vaccines ranged from 57.4% (7-valent) to 72.4% (23-valent) of all serotyped strains. This rise in pneumococcal resistance against penicillin and the macrolides and the shift in serotype in these resistant strains clearly warrant ongoing surveillance programmes in order to still be able to formulate both effective vaccination strategies and optimal antibiotic therapies in an era of ever-increasing resistance.

P1151 Tracking the activity of levofloxacin and comparator compounds against *Streptococcus pneumoniae* collected from five European countries during 2001–2002 and 2003

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Background: Continued increases in the prevalence of antimicrobial resistance among *S. pneumoniae* that are observed in many European countries demonstrate the importance of tracking resistance on an ongoing basis. The GLOBAL Surveillance initiative provides a unique perspective in the effort to track resistance among respiratory pathogens, with particular focus on tracking changes in the *in vitro* activity of levofloxacin (LEV), the most widely used respiratory fluoroquinolone.
Methods: During 2001–2002 and 2003, 5835 (2001–2002: 3915; 2003: 1920) *S. pneumoniae* were isolated from patient specimens collected at hospital laboratories in France (Fr), Germany (Ger), Italy (It), Spain (Sp), and the United Kingdom (UK). Isolates were centrally tested by broth microdilution against LEV, penicillin (PEN), azithromycin (AZI), ceftriaxone (CTX), and trimethoprim-sulfamethoxazole (TMP-SMX) (NCCLS, 2003). Susceptibility data

were analysed according to different parameters, including patient age and specimen source.
Results: During 2003, PEN R was 2.1% in Ger, 2.5% in the UK, 7.1% in It, 24.0% in Sp, and 31.9% in Fr. Fr showed the largest increase (5.6%) in PEN R compared with 2001–2002 (26.3%). AZI R was >20% in all countries except the UK (11.6%) with the highest rates reported in Fr (58.0%). Overall, LEV R was rare with an average of 0.98% and MIC90s = 1 mg/L in all countries. By age, LEV R was ≤0.8% among isolates collected from patients <18 years in all countries, 0–2.6% among isolates collected from patients 18–64 years, and 0–3.1% among isolates collection from patients ≥65 years. No isolates collected from blood specimens at laboratories in Fr, Ger, It, or the UK were LEV R; 2.2% of blood isolates in Sp were LEV R. Isolates collected from upper and lower respiratory specimens showed similar rates of LEV R with ranges of 0–1.1 and 0–2.6% in all countries, respectively.
Conclusions: LEV continued to show potent activity against *S. pneumoniae* despite increases in R to PEN and AZI. LEV was the most potent oral agent tested against *S. pneumoniae* collected from all age groups, including isolates collected from patients ≥65 years. Continued monitoring of antimicrobial resistance patterns is important to track changes in resistance, should they occur.

P1152 Antibiotic sensitivity and serotypes of *S. pneumoniae* isolates in blood culture during a 12-year period in a community-based hospital in Madrid, Spain

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Objectives: To find out the prevalence and seasonal distribution of pneumococcal bacteraemia and the serotypes and antibiotic sensitivity of isolates.
Material and methods: Retrospective study based on the records of our Microbiology Department with a focus on serotypes and MICs of pneumococcal strains isolated from adult and children with bacteraemia. Serotyping was performed by a Quellung technique in the National Reference Center. Sensitivity tests were performed by agar dilution following NCCLS criteria. Proportions comparison was performed by squared chi test.
Results: The average number of pneumococcal isolates in blood culture was 29/year which accounts for a 7% of all significant bacteraemias and a near 2/1000 admissions. A 62% of cases occurred from December to May. A total of 70% of patients were male. Age distribution was: 18.7% isolated from infants 0–2 years; 10.3% from children 3–15 years; 45.7% from patients 16–65 years; and 25.3% from patients >66 years. Nine serotypes (14, 19, 3, 6, 1, 8, 18, 9, and 4) account for near 75% of isolates. A total of 82% of serotypes were isolated from children under 2 years. Similarly, 96% of serotypes from patients >65 years are included in the polysacharide vaccine. The 33% of isolates showed a reduced sensitivity to penicillin (22% intermediate resistance and 11% resistant), 19% showed a reduced sensitivity to cefotaxime (16% intermediate resistance and 3% resistant). A 22% of isolates were resistant to erithromycin; 29% to tetracycline; 17% to cloramfenicol. All isolates were susceptible to vancomicine. Strains from children under 2 years were more resistant to antimicrobials, being this difference os statistical meaning ($P < 0.05$), standing out figures of 59% with reduced sensitivity to penicillin, 38% with reduced sensitivity to cefotaxime, and 43% resistant to erithromicin. No statistical difference was obtained between tetraciclina resistance proportion in the different age groups ($P = 0.215$).
Conclusions: In our environment, *S. pneumoniae* bacteraemia is found mainly in adult males. We found a high prevalence of strains with a certain degree of resistance to penicillin (33%), highlighting a 59% of resistant isolates from infants. The majority of serotypes from invasive infections are included in the current available vaccines.

P1153 Antimicrobial resistance among community-acquired respiratory tract infections in Brazil: PROTEKT 2002

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Objectives: Establish the susceptibility pattern of *S. pneumoniae* and the beta-lactamase production of *H. influenzae* and *M. catarrhalis* isolated from patients with community-acquired respiratory tract infections through an international surveillance study.
Methods: During 2002, six centres in Brazil collected bacterial isolates from patients with one of the following infections: pneumonia, acute bacterial exacerbation of chronic bronchitis, acute exacerbation of chronic obstructive airways disease, acute/chronic sinusitis, pharyngitis, and acute otitis media. *S. pneumoniae* isolates were tested against penicillin, amoxacillin, amoxacillin/clavulanic acid, cefuroxime axetil, cefaclor, and azithromycin. Minimal inhibitory concentrations (MIC) were determined by broth microdilution in a central lab. Interpretative criteria used were those described by NCCLS documents M100-S13. *H. influenzae* and *M. catarrhalis* isolates were tested for beta-lactamase production by chromogenic cephalosporin method (Cefinase[R]).
Results: There were 687 isolates as follows: *S. pneumoniae* (35%); *H. influenzae* (31%); *S. aureus* (15%); *S. pyogenes* (12%); *M. catarrhalis* (7%). Among *H. influenzae* and *M. catarrhalis* 10 and 100% were beta-lactamase producers, respectively. Among *S. pneumoniae* 67.6% were susceptible (S), 17.2% were intermediate resistant (I), and 15.1% were fully resistant (R) to penicillin.
Conclusions: The production of beta-lactamase among *M. catharralis* and *H. influenzae* strains was similar to that found by other local studies. The prevalence of penicillin resistant pneumococci has increased dramatically compared with previous local data, suggesting, among other factors, a possible methodological issues. Empiric therapy with penicillins alone or in low dose should be avoided in this population.

P1154 Study of invasive penicillin-resistant pneumococci isolated in Romania between 2000 and 2003

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Objective: To study the antibiotic resistance in pneumococci isolated in the past years in Romania.
Methods: 317 strains of *Streptococcus pneumoniae* coming from blood ($n = 196$) and CSF ($n = 121$) were collected between January 2000 and March 2003 at the National reference Center for Streptococcus. The isolates were tested for susceptibility (MICs) to the following antibiotics: penicillin (Pc), erythromycin (Em), tetracycline (Te), chloramphenicol (Cm), cephalothin (Kf), cefuroxim (Cxm), cefotaxim (Ctx), amoxicillin (Amx), trimethoprim/sulfamethoxazole (Sxt), ofloxacin (Ofx), vancomycin (Va) by standard dilution MIC testing.
Results: Breakpoints were used as proposed by NCCLS 2002. During the study period penicillin-resistant strains of *S. pneumoniae* were noted as follows: 40% in blood (25.9% low level and 14.1% high level) with MIC50 = 0.12 mg/L, MIC90 = 1 mg/L and 12% in CSF (9% low level and 3% high level) with MIC50 = 0.06 mg/L and MIC90 = 2 mg/L. The penicillin-resistant strains coming from blood showed 100% susceptibility to Amx and Ofx and resistance to the following antibiotics: Em (27%), Kf (36%), Cxm (27%), Ctx (9) against penicillin-resistant strains coming from CSF with following phenotypes: 100% susceptibility to Amx, Ofx, Ctx, Cxm, Cm and resistance to Em (33%), Kf (33%). No resistant strain to Va was found.
Conclusions: The most efficient drugs against penicillin-resistant pneumococci were: Amx, Ofx and Ctx. These results from Romania also underline the previous observations regarding the higher emerging rates of resistance in *S. pneumoniae* worldwide. In addition, the findings emphasise the importance of antimicrobial surveillance programmes for guiding empirical therapy.

P1155 Analysis of macrolide-resistant isolates of *Streptococcus pneumoniae* gained from the Far East of Russia

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Background: Macrolide resistance has been reported to be high among pneumococci in Asian countries, but the distribution of these macrolide resistance determinants is not known. The incidence of erythromycin-resistant strains among *Streptococcus pneumoniae* isolates in such a large territory as the Far East of Russia was approximately 5% until the early 1990s, but nowadays erythromycin-resistant strains have been greatly increasing.
Aims: To further define molecular mechanisms of macrolides resistance in pneumococci strains at the territory of the Far East of Russia.
Methods: MICs of penicillin, erythromycin, clarithromycin, and clindamycin were determined by the agar dilution method. PCRs were performed with appropriate primers.
Results: A total of 35.82% (48 of 134 strains) of the *S. pneumoniae* strains were resistant to erythromycin with an MIC of 1.0 g/mL. Of these, 31.25% (15 of 48) showed an MLSB phenotype with erythromycin and clindamycin 50% MICs (MIC50s) and MIC90s of 64 g/mL; 66.6% (32 of 48) showed resistance to erythromycin alone (M phenotype), with a MIC50 and MIC90 of 8.0 g/mL. One isolate was positive with both ermB and mefE primers. Of the isolates expressing the MLSB phenotype, only the ermB gene was detected in 86.63% (13 strains of 15) of the isolates by PCR. Two isolates were repeatedly negative on testing for ermB but were positive for ermA gene. All the isolates expressing the M phenotype were positive for the mefE gene by PCR. The majority of the M-phenotype strains (84.37%, or 27 of 32) had constitutive resistance (cML phenotype); only 15.625% of these strains had inducible resistance (iML phenotype). Before 2000, it was recorded (5) that among the erythromycin resistant *S. pneumoniae* isolates, the majority (78%) had an ML phenotype and 22% had an M phenotype. All *S. pneumoniae* isolates exhibiting a cML or iML phenotype harboured the ermB gene.
Conclusion: This study indicated a high percentage of erythromycin resistance among clinical isolates of S. pneumoniae in the Far East of Russia. It requires a more careful approach to diagnostics of macrolide resistance in pneumococci in the clinical microbiology laboratory, particularly in areas with high rates of macrolide resistance.

P1156 Impact of telithromycin vs. azithromycin on the nasopharyngeal microflora of patients with respiratory tract infections caused by pneumococci

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Objectives: Telithromycin belongs to the family of ketolides representing a new class of antibiotics. Telithromycin possesses a targeted spectrum of activity directed against pathogenic bacteria involved in respiratory tract infections. The purpose of the present study was to investigate the impact of telithromycin versus azithromycin on the nasopharyngeal microflora of patients with respiratory tract infections caused by pneumococci.
Methods: 227 patients from Germany with respiratory tract infections were enrolled in this prospective, assessor-blinded study and were randomised to telithromycin 800 mg od (5 days for upper respiratory tract infections, 7 days for pneumonia), or to azithromycin 500 mg od for 3 days. Nasopharyngeal and oropharyngeal cultures were taken from all patients before, during and after antibiotic treatment, i.e. at weekly intervals up to 42 days. The samples were cultured on different selective agar media and the microorganisms identified. In 50 of the patients, the presence of pneumococci was confirmed. These pneumococci were serotyped and the sensitivity to telithromycin and azithromycin was determined according to NCCLS. Additionally, clinical efficacy and safety was determined.
Results: Clinical cure was found in 95 of 113 patients treated with telithromycin and in 77 of 114 patients treated with azithromycin. Clinical failure occurred in eight patients (7%) in the telithromycin group and in 15 patients (13%) in the azithromycin group. Pneumococci were re-isolated in 5 of 28 patients (18%) in the telithromycin group and in 6 of 22 patients (27%) in the azithromycin group. The following pneumococcal serotypes were found: 3, 4, 6, 9, 11, 14, 15, 19, 20 and 23. All (100%) pneumococci were sensitive to telithromycin, MIC range 0.008–0.5 mg/L; 12% of the pneumococci were resistant to azithromycin, MIC range 0.064–512 mg/L. A variety of other microorganisms, i.e. staphylococci, streptococci, enterococci and candida, were isolated. Both drugs were well tolerated.
Conclusions: The patients treated with telithromycin had a better clinical response than the patients treated with azithromycin. The isolated pneumococci were more susceptible to telithromycin than to azithromycin. Furthermore, telithromycin appeared to have a more favourable ecological and efficacy profile compared with azithromycin in these patients with pneumococcal infections of the respiratory tract.

Antibiotic therapy and public health issues

P1157 Process costs of intravenous antibiotic therapy for community-acquired intra-abdominal infections

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Objective: A time and motion study was conducted in a large academic hospital in Lund, Sweden to assess the actual time and supplies involved in the preparation, administration, and routine monitoring of IV antibiotics to patients admitted to the hospital with an IAI requiring IV antibiotic treatment and to estimate the cost of the total process of preparing and intravenously administering an antibiotic from the perspective of the hospital, separately from the acquisition cost of the antibiotic.
Methods: Routine acts of preparing and administering cefuroxim (Zinacef®) by gravity-driven (piggyback) intermittent infusion, or by bolus injection for treatment of community-acquired IAI were observed and timed by a research nurse at the general surgical ward of Lund University Hospital between September and November of 2003. Also, materials consumed, other than the antibiotic, were recorded. Cost of administration was calculated based on personnel time and materials consumed multiplied by respective salary and purchase price for different materials (expressed as 2003 SEK).
Results: A total of 60 observations were made, 31 for piggyback administration while 29 for bolus IV administration. The average overall time for each IV administration was 8.70 min (SD 2 min 29 s), which included review of chart/prescription (average time ± SD: 0.72 ± 0.44 min), preparation of drug (3.27 ± 1.26 min), administration of drug (4.35 ± 1.28 min) and disposal of materials (0.36 ± 0.17). The average overall time for preparation and administration of each piggyback IV administration was 8.99 min (SD = 2.08) while that for bolus IV administration was 8.38 min (SD = 2.13). The average overall cost of materials used excluding cost of drugs in each administration was 23.57 SEK (29.80 SEK for piggyback and 16.91 SEK for bolus), while average overall labour cost was 35.25 SEK (36.44 SEK for piggyback and 33.97 SEK for bolus). Thus the average cost of each IV antibiotic administration was estimated to be 58.82 SEK (SD = 13.04) while the acquisition cost of the study drug was SEK 74.80 for a 1.5 g vial and SEK 34.40 for a 750 mg vial.
Conclusions: The cost of each intravenous antibiotic administration including personnel time and material cost involved in administering and preparing the IV antibiotic approaches the cost

of antibiotic. Simplifying drug regimens and methods of administration may reduce these components and lead to considerable cost savings.

P1158 Economic impact of *S. pneumoniae* macrolide resistance in children with acute otitis media in five European countries

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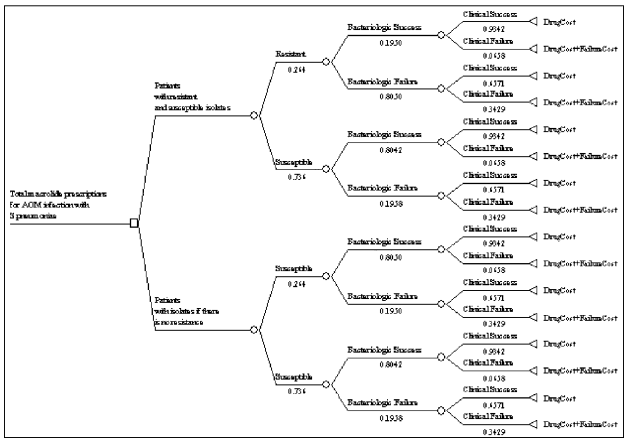
Objective: To estimate the economic impact of *S. pneumoniae* (SPN) macrolide (MAC) resistance in children with AOM, in five European countries using resistance rates (RR) from the Alexander Network 2001 (AN); randomised clinical trial (RCT) data containing: erythromycin-based RR, bacteriologic and clinical success and failure rates; and published literature.
Methods: Medline and IPA were searched for country-specific estimates of incidence and number of MAC prescriptions, for children with AOM. Country-specific SPN MAC RR were obtained using isolates from the AN. Data from the azithromycin arm of a RCT was used to obtain the increased risk of bacteriologic failure in SPN MAC-resistant strains by comparing patients with resistant vs. susceptible isolates. Increased risk of clinical failure associated with bacteriologic failure was also obtained from the RCT. Data 4.0 (TreeAge, Inc.) was used to create a decision tree, shown below. Drug costs, based on average wholesale price, were obtained from the Genesis and Europrice databases. Treatment failure costs include new drug and one office visit (MedTap International Database of Unit Costs for Health Care 2001).
Results: In the RCT, children with SPN MAC resistant strains had an increased risk of bacteriologic failure vs. MAC susceptible strains, relative risk = 4.11 (95% CI 1.76–9.63). France and Italy had higher treatment failure costs per case, 16.35E and 9.56E, respectively. Germany and the UK had lower RR and thus lower treatment failure costs, 2.45E and 2.01E per case, respectively. Remaining results are presented in the table below.



Country	AOM Incidence Rate* ($\times 10^6$)	Percentage of AOM Rx's for a MAC	Approximate AOM Cases using MAC's# ($\times 10^3$)	AN RR for MACs	Total Estimated Population Cost ($\times 10^6$) 2003 Euros		
					Cost of Resistance		
					Res. & Susc.	No Resistance	Difference
France	0.82	23.0%[3]	70.5	57.7%	3.45	2.30	1.15
Germany	1.22	48.1%[2]	220	11.4%	8.01	7.47	0.54
Italy	1.14	40.1%[1]	172	35.9%	5.11	3.47	1.64
Spain	0.57	10.1%[4]	21.5	26.4%	6.64	5.20	0.14
UK	1.11	12.9%[5]	53.5	11.1%	1.38	1.28	0.11

*Miller *et al.* UK-based incidence rate
#Correction factor of 35% accounts for *S.pneumono* prevalence
1. Borgnolo G, *et al.*; 2. Schindler C, *et al.*; 3. Gulleimon D, *et al.*; 4. Solis G, *et al.*; 5. Molstad S, *et al.*

Conclusions: MAC resistance can lead to increased bacteriologic and clinical failure rates. Countries with higher RR appear to have higher failure costs if MACs are commonly prescribed. In terms of economic costs, high failure rate may have significant implications for health care payers.

P1159 Comparison of level of physician satisfaction with ertapenem, piperacillin/tazobactam and ceftriaxone/metronidazole for the treatment of community-acquired intra-abdominal infections requiring surgery

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Objective: To compare the level of satisfaction with ertapenem (ERT, a once-a-day parenteral group 1 carbapenem) to piperacillin/tazobactam (P-T) and ceftriaxone/metronidazole (C-M) in the treatment of IAI requiring surgical intervention.
Methods: In two open-label, multicentre trials (OASIS I and II), 802 adults with clinical and/or radiographic evidence of IAI who required surgery were randomised to ERT 1 g, P-T 13.5 g daily, or ceftriaxone 2 g daily (2 g IV/IM q24h or 1 g IV/IM q12h) and metronidazole 30 mg/kg daily (q6h, q8h or q12h, IV or oral) daily for 4–14 days. Physician's satisfaction with over all therapy, number of times of administration the study antibiotics, efficacy of the study antibiotics, and safety and tolerability profile of the study antibiotics were rated at discontinuation of therapy visit on a seven-point scale where 1 indicates very satisfied and 7 stands for very dissatisfied. One-way analysis of variance between group designs was used to compare the mean satisfaction with each of these three treatments. The null hypothesis was that there were no differences between the three treatment groups with respect to their mean scores of the satisfaction. When the null was rejected, pair wise comparisons were made after adjusting for multiple comparisons using Tukey's HSD test.
Results: Baseline characteristics were comparable in all treatment groups. The mean scores of physicians' over all satisfaction with therapy were 1.53, 2.04 and 1.76 for ERT, P-T and M-N, respectively indicating significantly higher level of overall satisfaction with ERT ($P < 0.05$). Similarly mean scores of physicians' satisfaction with safety and tolerability, efficacy profile, and number of times of administration of treatment were significantly ($P < 0.05$) lower for ERT as compared with P-T and C-M (see table) indicating higher level of satisfaction with ERT.

Satisfaction with	Mean satisfaction score		
	Ertapenem ($n = 397$)	Piperacillin/ Tazobactam ($n = 188$)	Ceftriaxone/ Metronidazole ($n = 217$)
Safety and tolerability	1.55	1.85	1.74
Efficacy profile	1.54	1.83	1.72
Number of times of administration	1.32	2.82	2.23
Overall therapy	1.53	2.04	1.76

Conclusion: Physicians were significantly more satisfied with ertapenem as compared with piperacillin/tazobactam or ceftriaxone/metronidazole in treating IAI patients requiring surgery. This includes higher level of satisfaction with safety and tolerability, efficacy profile, and number of times of administration of ertapenem and overall satisfaction with ertapenem.

P1160 Fixed-dose combination drugs for tuberculosis and HIV/AIDS: what are the issues?

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Objective: To determine issues of concern on the use of fixed dose combination (FDC) drugs for tuberculosis (TB) and human immu-

nodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS).
Methods: The United States Pharmacopeia (USP), a not-for-profit, non-governmental organisation, has been the standard setting authority for pharmaceuticals in the US since 1820. Through its Drug Quality and Information (DQI) program, USP conducted a literature search and reviewed studies and reports published between 2000 and 2003 on FDC drugs used in the treatment of TB and HIV/AIDS.
Results: For anti-TB drugs, the bioavailability of rifampicin in several marketed FDCs containing isoniazid and/or pyrazinamide has been demonstrated to be inferior/variable, which could result in therapy failure; it also contributes to the increasing resistance to anti-TB drugs. FDCs available in strip-packed products were more stable than blister-packed products which showed physical and chemical changes; unpacked products showed a 60% decomposition of rifampicin. Limited stocks of single-drug TB tablets need to be kept for those experiencing adverse events with FDCs. For HIV/AIDS drugs, some patients on FDCs may experience side effects caused by one of the medicines in the combination product; packaging the drugs in one tablet would limit the flexibility to switch patients to a different drug combination. The International Federation of Pharmaceutical Manufacturers contends that generic FDCs for HIV/AIDS have not been rigorously tested which may encourage counterfeit drugs to enter the market. Paediatric equivalents, especially crucial now that more paediatric AIDS cases are being diagnosed due to increasing number of mother-to-child transmission (MTCT) interventions being carried out are seriously lacking.
Conclusions: There are a number of concerns with the use of FDCs that need to be addressed. Quality assurance of FDCs for TB is needed through bioavailability testing by manufacturers. Packaging materials used for FDCs should be of the highest quality. Paediatric formulations, especially for FDCs used for HIV/AIDS, should be developed. (Splitting FDC adult tablet is unacceptable, but in the absence of alternatives, health care providers in resource-limited settings are left with no choice.)

P1161 Association between inappropriate initial empiric antibiotic therapy and outcomes among patients undergoing surgery for community-acquired intra-abdominal infections in Israel

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Objective: To assess the association between inappropriate initial empiric antibiotic therapy and the clinical outcome among patients undergoing surgery for community-acquired intra-abdominal infections (IAI) in Israel.
Methods: Records of patients who underwent surgery for community-acquired IAI from January 2000 to June 2002 in hospitals in Israel were reviewed. Initial empiric antibiotic therapy was classified as inappropriate if at least one pathogen was resistant to all antibiotics in initial regimen in case of positive culture or not according to guidelines in case of negative/missing culture. Therapy was classified as successful if IAI was resolved with initial therapy or with decrease from initial therapy; as unsuccessful otherwise. Logistic regression analyses were performed to assess associations between inappropriate therapy and clinical outcome, after adjusting for patients' characteristics and site/type of infection.
Results: 279 patients were included. Mean (SD) age was 53.2 years (21.05) while 36.8% were female. Almost 87% of the patients received appropriate initial empiric therapy, while 75.9% of all patients' initial empiric therapy was successful. Compared with patients on appropriate therapy, patients on inappropriate therapy were less likely to have IAI resolved with initial therapy (45.9 vs. 80.2%, $P < 0.0001$). Multivariate logistic regression showed that patient who received inappropriate initial empiric therapy were almost four times less likely to have success in their therapy (OR = 3.6, 95%CI: 1.6–7.8).
Conclusion: Among patients undergoing surgery for community-acquired IAI in Israel, inappropriate initial empiric antibiotic therapy significantly increases the likelihood of unsuccessful clinical outcomes.

P1162 Social and financial factors influencing rational use of antibiotics in Peru

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Objective: To investigate social and financial factors influencing antibiotic use in households with children in an urban community in the jungles of Peru.
Methods: Cross-sectional study including household interviews and focus group discussions (FGD) in the urban community Yurimaguas, Peru. Carers of 800 children aged 6–72 months were interviewed on health seeking strategies (public/private sectors; formal/informal providers) and antibiotic use (type, number of days, cost, administration, expiry date, etc.) in relation to reported symptoms and socio-economic status. 15 FGDs (6–8 participants/group) were performed with health workers and caregivers, including mothers, fathers and grandmothers (topics discussed include concepts and utilisation of pharmaceuticals and traditional medicine, gender and opinions on antibiotics and health services).
Results: 36% of the children with symptoms reported use of antibiotics within 2 weeks, most frequently for acute respiratory infections (ARI) and diarrhoea-like syndromes. Many children obtain health care and medicines free of charge, due to the new public health insurance. Self-medication with antibiotics for children is low. Use of traditional medicine is frequent. Doubts about quality of 'insurance-antibiotics' and lack of information on proper use of antibiotics contribute to problems with compliance. Health seeking behaviour and antibiotic use vary with socio-economic status. Different types of irrational use of antibiotics are found to be linked with different socio-economic groups of the society.
Conclusions: The new public health insurance has increased access to health care for children. Interventions are recommended to address health care providers and include providers–patient dialogue addressing topics related to rational use of antibiotics. Community interventions should promote antibiotic compliance and home management of symptoms including appropriate use of traditional medicine. Interventions acknowledging the different types of irrational use of antibiotics among different societal groups will be more prone to succeed.
Acknowledgements: EC-INCO DEV for RTD projects, 5th framework programme of the European Community for research. Contract number: ICA4-CT-2001-10014.

P1163 Antibiotic consumption trends in the case of fever with unknown aetiology in the Turkish community

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Objectives: Appropriate antibiotic consumption defined as right diagnosis, clinically maximum impact for treatment, minimum side effect, prevention of antibiotic resistance and cost-effectiveness. Antibiotic consumption principles are same with other drugs. Bacterial resistance develops because of inappropriate consumption of antibiotics and affects not only the patients' but also the community's health. Multi-drug resistance results in unsuccessful treatment of bacterial infections and because of these resistant species are widespread, selective resistance rates increases throughout the world. The community should be informed about the appropriate antibiotic consumption.

Methods: Dokuz Eylul University Hospital is a 720-bed tertiary care hospital. From January 2000 to June 2003, 850 patients were hospitalised in Infectious Disease Clinic. Of them, 79 had no symptoms except fever. Patients who presented with only fever were questioned for antibiotic use, antibiotic choice, dosage, dosing interval, and duration of therapy.
Results: Out of 79 patients, 46 were male (58.2%) and 33 were female (41.8%). Age interval was between 18 and 93. Of the patients 38 (48.1%) have taken no antibiotics, 41(51.9%) have used at least one antibiotic before hospitalisation. Of 41 patients, 22 of them had one drug, 8 had 2 drugs, and 2 had 4 drugs. Amoxicillin-clavulanate and levofloxacin were the most common antibiotics used. According to final diagnosis of patients who used antibiotics before the admission, 32 (78.1%) of them had no indication for antibiotic use while the others (9–21.9%) had bacterial infections and necessity of antibiotic usage. Of the 9 patients who had indication for antibiotic therapy, 4 patients have taken inappropriate therapy. Of 38 patients who came up to hospital without having any therapy, 15 (39.5%) had antibiotherapy indication and treated with appropriate drugs. Of 38 patients 23 (60.5%)had no diagnosis of infectious aetiology. It was also observed that most of the patients having antimicrobial therapy have not used drugs appropriately according to dosage, dosing interval and therapy period.
Conclusions: In our study we concluded that inappropriate and unnecessarily usage of antibiotics is common in our community. In order to prevent this problem, community and healthcare workers have to be informed about antibiotics policy programmes.

P1164 Analysis of urgency and effectiveness of post-graduate distance education on antimicrobial therapy in Russia

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Objectives: (1) To assess the urgency of development of post-graduate distance education (DE) programme on antimicrobial therapy (AT) in Russia. (2) To evaluate effectiveness of DE course upon changes of 'students' level of knowledge after completion of DE course in comparison with baseline.
Methods: First Internet centre of DE in Russia was founded under the auspices of the Institute of Antimicrobial Chemotherapy, the Department of Clinical Pharmacology of Smolensk State Medical Academy with the support of the United States Pharmacopeia and the United States Agency for International Development. Web-site for medical professionals 'Antibiotics and Antimicrobial Therapy' (http://www.antibiotic.ru) serves as the basis for this DE Internet centre. To assess the urgency of DE for physicians the on-line survey was performed on www.antibiotic.ru. To evaluate the effectiveness of DE course the comparison of baseline level of knowledge in AT and the final test (after completion of the DE course) results of 'students' was done.
Results: 491 of www.antibiotic.ru visitors took part in the on-line survey 'Urgency of DE (via Internet) for physicians'. 309 respondents (63%) answered 'Yes, definitely!', 57 (11.6%) – 'Yes, probably', 48 (9.8%) – 'Doubtfully, Internet access is not available for everyone', 67 (13.7%) – 'No, physicians are not ready to learn via Internet' and 10 (2%) – 'I don't know, I am not a physician'. Since the implementation of the project, 87 doctors from 12 Russian regions have been trained using DE technology. Analysis of the DE results revealed the obvious increase in the number of the correct answers given by physicians in their preliminary test (62.6%) and the final exam (88.7% of correct answers), confirming the effectiveness of the DE. After passing the final examination all physicians received the official state certificate confirming the improvement of knowledge in the field of AT.
Conclusions: (1) DE via Internet is a demanded form of post-graduate professional education among physicians in the field of AT in Russia. (2) DE is an effective form of professional level increase in AT.

P1165 Impact of an antibiotic policy in a community hospital

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Objective: To evaluate the impact of an antibiotic policy on the antibiotic usage and costs.
Methods: In March 1999 an antibiotic policy was implemented in a 320-bed community hospital. The main strategies used were: (1) Constitution of an antibiotic committee (two pharmacists, two infectious diseases physicians [IDP], and a microbiologist). (2) Elaboration of a guide to empiric antimicrobial therapy for the prevalent community-acquired infections, after consensus meetings with the concerned services. (3) Elaboration of a controlled antibiotics list. (4) No prescribing restrictions. (5) Daily revision by an IDP of the controlled antibiotics prescribed in the hospital, and personal discussion with the prescribing doctor when deemed appropriate. The average time devoted to the programme by the IDP was 10 h weekly. During the 1998–2002 period, the annual antibiotic consumption was recorded by the pharmacy. The data are expressed as defined daily doses (DDD) per 100 bed days. The annual antibiotic costs were also calculated.
Results: From 1998 to 2002 there was an increase in the use of amoxicillin (+3.03 DDD/100), amoxicillin/clavulanate (+31.24 DDD/100), 1st generation cephalosporins (+1.79 DDD/100), penicillin (+0.52 DDD/100), carbapenems (+0.43 DDD/100), and quinolones (+2,53 DDD/100), and a decrease in the use of 2nd generation cephalosporins (−11.62 DDD/100), macrolides (−2.69 DDD/100), 3rd and 4th generation cephalosporins (−2.25 DDD/100), and aminoglycosides (−0.55 DDD/100). There was no significant modification in the use of glycopeptides (+0.07 DDD/100) or piperacillin/tazobactam (−0.17 DDD/100). From 1998 to 2002, the overall expenditures for antibacterial agents decreased every year, reaching a 26.5% reduction in 2002, in spite of a 13.5% increase in the total bed days during the 5-year period.
Conclusions: The implementation of an antibiotic policy results in a significant change in the antibiotics usage. The overall expenditures for antibacterial agents decreased by 26.5% in a 5-year period.

P1166 Costs of MRSA infection in a department of physical medicine and rehabilitation

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Objectives: To determine the additional costs involved in dealing with MRSA infection or colonisation in a department of physical medicine and rehabilitation.
Methods: At a Munich academic teaching hospital with 1000 beds and with an MRSA incidence of 13% (expressed as the percentage of the total number of penicillin resistant *Staphylococcus aureus* : $n = 1228$ for 2003), the actual costs relating to MRSA infection per patient were prospectively studied in the department of physical medicine and rehabilitation. After patients had been identified with MRSA infection in the 38-bed unit, the recommended procedures (as stipulated by national and local recommendations) to deal with MRSA were put into place. The procedures were prospectively documented on check-lists each time upon entry into the patient's single isolation room for the duration of the patient stay. This included amongst others time to put on or discard gloves, masks as well as gowns, once daily floor disinfection by the cleaning staff, time to relate the MRSA issue to family members and friends, time to perform therapeutic measures (intravenous vancomycin, linezolid, intranasal mupirocin), time to perform diagnostic measures (nose, axilla and groin swabbing), time to perform logistical measures and disinfection of used materials. The time required for performing each procedure was defined by the infection control sister as an average of 10 timed procedures. These were then expressed in monetary values (€) relating to staff – equipment – and other costs. Costs of bed closure were also included.

Results: The procedures to deal with MRSA infection (single bedroom, gowning, gloving and masks if required has led to considerable extra monetary costs compared with the non-infected patient in long-lying patients. On average, the staff have to gown up 20 times per day per patient upon entry in to the room. To move existing patients around to other rooms in the ward when a new MRSA case is admitted to a single room necessitate logistics, which are very time consuming.
Conclusion: MRSA infection causes significant costs in terms of time, increased need for staff and therapeutic costs. In Germany reimbursement of patients with MRSA infection is not adequately represented in the current diagnosis related group (DRG) reimbursement scheme, which is in operation since the beginning of 2004.

P1167 Evolution of the institutional antibiotic policies between 1999 and 2002 in French hospitals

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Objective: To determine the evolution of the institutional policies to control use of antibiotics and to improve antimicrobial use.
Participants and method: Pharmacy and infection control staff at 37 hospitals participating in the survey conducted by the CCLIN SO. We collected information about total antibiotics used and about susceptibility of *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolates recovered from clinical specimen during 12 consecutive months, as well as descriptions of the type of antibiotic management policies and procedures being used by the individual hospitals by means of a standardised questionnaire in 1999 and in 2002. Responses were analysed using chi-square MacNemar and Wilcoxon matched-pairs signed-ranks test.
Results: 37 hospitals with a median of 205 beds (range 28–893) and with a mean of 54 029 patients-days in 2002. Respectively, in 1999 and in 2002: a local committee supervised antibiotics use in 47 and 80% of the hospitals ($P < 0.01$), 80% had a validation process before dispensing antibiotics. Local clinical practice guidelines were reported at 29.7 and 48.6% of the hospitals, feed-back information existing in 51 and 83.7% ($P < 0.01$) for antibiotic consumption and in 57 and 86.5% for antibiotic resistance ($P < 0.01$). Fewer than 30% used computer for the management of dispensing of antibiotics. Only 3% used an electronic network to share information on antibiotics prescription and bacteriological results. Fewer than 30% reported evaluation of antibiotic practices. Decreasing in antibiotics consumption was not significant: respectively, 364 and 345 defined daily doses per 1000 patients-days (DDD/1000). In 2002, a lower antibiotic consumption was associated significantly with antibiotic order form obligatory for getting the drug from pharmacy (441 vs. 305 DDD/1000). Variations of bacterial resistance rates were not significant for *Staphylococcus aureus* and methicillin, *Pseudomonas aeruginosa* and imipenem or ceftazidime or ciprofloxacin.
Conclusion: Our survey shows improvement in the reported antimicrobial use programmes but hospitals also must focused on appropriateness of use and on computer equipment.

P1168 The first 3 years of an outpatient and home parenteral antibiotic therapy (OHPAT) service in Glasgow

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Objectives: To evaluate how patterns of referral and patient management strategies have evolved over the first 3 years of the Glasgow OHPAT service.
Methods: Comparisons were made between years 2001, 2002 and 2003 (until December 8th) utilising patient data recorded prospectively.
Results: Since January 2001, 588 patients have received OHPAT (112, 236 and 240 per year). Growth reflects increasing referrals from A&E (6, 70, 57 per year), GP (3, 18, 37 per year), medicine (16, 61, 46 per year). Referrals from ID (55–63 per year) and orthopaedics (13–21 per year) were similar. Increasing numbers of patients with skin and soft tissue infections (62 (55.4%), 174 (74%) and 154 (62%), bone and joint infections (BJI) (22 (19%), 34 (14%) and 37 (16%)), endocarditis/ bacteraemia (7 (6%), 10 (4%), 16 (7%)) and MRSA infections (10 (9%), 20 (9%), and 33 (14%) were observed. Admission was avoided in 28 (26%), 116 (49%) and 137 (57%). The number of days of OHPAT service for each year was 1814, 2856 and 2913 days with a median length of therapy per patient of 9, 5 and 5 days (range 1–107). A similar proportion of patients self-administered therapy (10, 7, and 12%), although a greater proportion with BJI self-administered in year 3 (30 vs. 4% in year 1 and 18% in year 2). Fewer patients with BJI received home visits in year 3 (5 vs. 55% in year 1 and 19% in year 2). Unplanned admissions attributed to the infection or the therapy occurred in 5, 4 and 8% of patients. Cure or improvement at end of treatment was observed in 95, 93 and 97%. Overall three deaths occurred and none were directly attributable to the infection or therapy.
Conclusions: The service in Glasgow is expanding and providing a safe alternative to hospital admission for a wide range of patients with infection. Increasing numbers of patients with soft tissue infection are referred directly from A&E or GP and are managed without admission. More patients on longer-term therapy are self-administering antibiotics at home.

P1169 Antibiotic prescribing knowledge among junior doctors – how can we improve it?

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Background: Prescribing of antibiotics, often in the empiric setting, frequently falls on training grade or junior doctors, who are often the least experienced in this. Recognition of infection and sepsis represents basic components of good antibiotic prescribing practice.
Methodology: We undertook two questionnaire and interview based surveys (1999 and 2003) of prescribing training grade doctors in a teaching hospital in North-East Scotland to determine their knowledge level regarding (i) doctor's knowledge of the definitions of systemic inflammatory response syndrome (sirs), sepsis, severe sepsis and septic shock; (ii) the source of local or other information each doctor used when prescribing an antibiotic; (iii) the criteria each doctor used when choosing the route of administration of antibiotic and to determine if each doctor knew the likely cost differential between an intravenous and oral antibiotic. Interventions: Between the two audits 4 years apart several initiatives have been introduced to improve the education and support related to antibiotic prescribing. These included a pharmacy and ID led 6-monthly teaching programme for all residents and final year medical students in Dundee, introduction of sepsis protocols on all medical and surgical wards and improving access to and awareness of the local antibiotic prescribing formulary.
Result: (see attached table/figure).

Table 1. Results of the antibiotic prescribing knowledge among junior doctors

					IV route/source of information			Cost awareness difference between IV and oral
Audit year	% sirs	% sepsis	% severe sepsis	% septic shock	Sepsis protocol	Micro/ID advice	BNF	
1999	21%	38.2%	38.2%	20%	10.9%	47.2%	23.6%	36.3%
2003	65.4%	54%	48%	67.4%	40%	26.7%	2.7%	35.8%

% indicate positive or correct responses for the above definitions or criteria BNF = British National Formualry

Fifty-five junior doctors in a large teaching hospital participated in the survey in 1999 and 78 in 2003. There has been a significant improvement in doctor's knowledge of regarding various sepsis definitions and more (~29%) used the desired locally derived sepsis protocol, which guided the prescriber through sepsis recognition, empiric choice and monitoring of antibiotics. Use of this protocol appeared to be at the expense of telephone ID/micro advice and the BNF, which we regard as not ideal as a local sepsis management guideline. In term of awareness of the cost difference between intravenous (IV) and oral antibiotic there was no significant improvement in the percentage of doctors that could acknowledge that parenteral antimicrobials were more expensive than oral antimicrobials (63.7% in 1999, 64.2% in 2003). Overall, there was significant improvement in doctor's knowledge in understanding sepsis and the source of information they utilised to select the antibiotic of choice although the majority did not acknowledge the price difference between IV and oral forms of antibiotics.

P1170 Antibiotic prophylaxis in severe acute pancreatitis: randomised multicentre prospective trial with meropenem

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Background: Nowadays, most deaths in acute pancreatitis are caused by infection, thus the role of prophylaxis of infectious complications in severe acute pancreatitis (SAP) still remains a debatable issue.
Aim: The aim of the study was to assess the efficacy of prophylactic antibiotic treatment comparing rates of local and infectious complications, mean hospital stay, necessity of surgical treatment and mortality in two groups of patients with SAP.
Methods: 41 patients with SAP were enrolled into the randomised multicentre prospective study according to the clinical criteria defined at the Atlanta symposium, necrotising pancreatitis on CT or CRP level exceeding 190 mg/L. The patients were randomised either into the prophylactic (P) or into the therapeutic (T) group. In the P-group, meropenem 500 mg was administrated every 8 h for 10 days. In the T-group, the patients received the same antibiotics only in the case of confirmed infectious complications (infected necrosis, abscess, urinary infection, pneumonia, etc.).
Results: The most important assessed criteria were: mean hospital stay, local complications, infectious complications, surgical treatment and mortality. No statistically significant difference between group P and group T was found regarding the examined criteria.
Conclusions: Prophylactic antibiotic treatment reduced neither the rate of infectious complications nor mortality and other criteria in patients with SAP.

P1171 Inventory and identification of lactic acid bacteria used as probiotics

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Objectives: Within the framework of the European project PROSAFE – Biosafety evaluation of lactic acid bacteria used for human consumption – we made an inventory of commercial probiotic strains, verified their identification and collected relevant information.
Methods: Fifty-four companies involved in the production and/or distribution of probiotics were invited to submit their strains and to complete a questionnaire. Species identification of the strains was verified using amplified fragment length polymorphism (AFLP), repetitive DNA element (rep)-PCR fingerprinting, and protein profiling.
Results: Of the 54 companies contacted, 27 submitted their strains, 13 claimed not to manufacture probiotics and were therefore excluded from the survey, 2 did not wish to participate, and 12 (mostly US companies) did not respond. All 27 participating companies returned the questionnaire. In total, 202 strains were submitted and received as Lactobacillus (54.0%), Bifidobacterium (26.7%), Enterococcus (5.9%), Propionibacterium (5.9%), Lactococcus (2.5%), Pediococcus (2.0%), Streptococcus (2.0%), Bacillus (0.5%) and Oenococcus (0.5%). The most frequently used identification techniques included biochemical characterisation (34.9%), DNA fingerprinting (21.8%) and 16S/23S rDNA sequencing (20.8%). Comparison with our current identification results for 174 strains, the identity of 17.2% of these strains did not correspond to the identification by the company. Out of 202 strains, 53.5% are of human origin, 44.5% of non-human origin, whereas for 2.0% the source of isolation is unknown. Two strains were submitted as genetically engineered. 46.5% of the strains are used for human consumption, 5.4% for animal use, 7.0% for both human and animal use, 7.4% were categorised as probiotic, 5.0% are industrial starters and 28.7% are still under investigation.
Conclusions: A large number of strains received from the contacted companies was correctly identified and the majority belonged to the genera Lactobacillus and Bifidobacterium. More than half of the strains are of human origin, and about the same number is used for human consumption.

P1172 Implementation of quality assurance programme for manually cleaned bronchoscopes following a pseudoepidemic of *Mycobacterium chelonae*

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Objectives: To standardise cleaning and high-level disinfection protocols for manually cleaned bronchoscopes in a tertiary care institution. To develop a quality assurance program that tracks the usage and reprocessing of bronchoscopes.
Methods: Six bronchial lavage samples yielding acid-fast bacilli in a 3-week period from a critical care trauma unit lead to an investigation of manually cleaned bronchoscopes. Environmental samples and rinse water from bronchoscopes post-disinfection were cultured for 7 days at 30°C. The acid-fast rods were identified using high performance liquid chromatography (HPLC) and typed using an adaptation of the Enterobacterial Repetitive Intergenic Consensus Sequencing (ERIC) method. A quality assurance method was implemented and correctional measures were made in the processing of bronchoscopes.
Results: Percentage of the six patient isolates and three environmental samples were identified as *Mycobacterium chelonae*. The sixth was identified as *M. fortuitum/peregrinum*. The molecular patterns for *M. chelonae* were identical. A contaminated hose filter used to drain the bathes was identified as the point source. Weekly cultures results of water rinsed bronchoscopes post-disinfection yielded reports ranging from sterile to mixture of bacteria including acid-fast rods, and fungi.
Conclusions: Many hospitals are cleaning and disinfecting bronchoscopes manually in less than ideal conditions. Inadequately trained personnel and poor quality control pratices may lead to contaminated bronchoscopes. It is recommended that infection control personnel review the cleaning and disinfection procedures and that a quality assurance method include proper documentation and chemical and or biological testing to ensure the safe reuse of bronchoscopes.

P1173 Occupational sharp injuries and exposure to body fluids among health care workers

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Objective: To determine the risk factors of sharp injuries and exposure to body fluids among health care workers (HCWs).

Method: The study was conducted in the hospitals of Ankara University School of Medicine. The maximum number of HCWs was targeted to be included in the study. The study was approved by the Research Board of the hospital. All the HCWs were asked to participate in the study, and the HCWs, who had accepted to participate were included. A structured survey form was administered by person to person interview. The survey included the questions related to demographics, injury, attitude of HCW after injury, and status of vaccination against Hepatitis B. The logistic regression was modelled to determine the risk factors for the needlestick injury occurred in the last 6 months.
Results: The study included 988 HCWs. The mean age was 31, and 70% were female. The study group included 500 nurses (51%), 212 residents (21%), 152 nurse assistants (15%), 26 radiology technicians (2.5%), 46 laboratory technicians (4.5%), 36 fellows (3.5%), and 16 anaesthesiology technicians (2.5%). The majority of HCWs (68%) had been vaccinated against hepatitis B before. The rate of the exposed (sharp injuries and exposure to body fluids) HCWs was 64%. The most frequent causes of the exposures were, recapping the needle (38%), operation (15%), phlebotomy (10%), suturing (5.2%), and resuscitation (4.6%). Of the injured HCWs 211 (33%) were not using protective equipments (masks, gloves, etc). The most common (55%) reason for not using protective equipment was being in a hurry. The number of the injured HCWs who did not seek any medical advice for injury was 381(61%). The age, gender, and duration of work had no effect on the needlestick injuries. However, being a nurse (odds ratio 2.3, confidence interval, 1.2–4.5, $P = 0.015$), and working overtime (OR, 1.3; CI, 1.1–1.6; $P = 0.015$) affected the outcome.
Conclusion: Majority of HCWs had the history of sharp injuries. Nurses working overtime had higher risk of needlestick injuries. Systematic occupational exposure control programmes should be implemented.

P1174 Multicentre microbiological trials based on non-profit membership framework

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Objectives: To determine optimal type of organisation structure for multicentre microbiological trials.
Methods: Official reports of different scientific non-profit organisations and published data on management of similar organisation were analysed.
Results: Since 1997, Interregional Association for Clinical Microbiology and Antimicrobial Chemotherapy (IACMAC) with the active participation of 1,897 members (33 regions of Russia) has been conducting the monitoring of antimicrobial resistance in Russia. That monitoring has some advantages compared with similar activity of profit and state research organisations: (a) It is not merely well-paid work, but great scientific interest that is a motivation for member-researchers' activity on antimicrobial resistance monitoring. (b) Collection of data on antimicrobial resistance is a result of close communication between members and leaders of IACMAC, which is a result of joint scientific and educational actions as well as functioning Internet portal http://www.antibiotic.ru. (c) Reliability and validity of data being obtained is due to high authority of IACMAC, using social marketing concept and principles of evidence-based medicine. Dependence of IACMAC on sponsors (pharmaceutical companies) is minimal, whereas the level of collaboration and mutual confidence between IACMAC and international societies, such as APUA, ISC, ESCMID, and FESCI is very high. The IACMAC took part in 11 multicentre microbiological studies on antimicrobial resistance of community-acquired and nosocomial pathogens in Russia. With the international support, 55 000 copies of 'Practice guidelines on antimicrobial chemotherapy' were published and distributed free of charge to the medical community.
Conclusions: As an example of successful scientific activity, IACMAC is an association that forms necessary public awareness about resistance among professionals and non-medical opinion leaders, and develops national policies in the field. Results of IACMAC activities over the past 5 years demonstrate the advantages of researchers collaboration within a non-profit organisation.

P1175 Comparison of the different disinfecting capacities of contact lens solutions for rigid gas permeable (RGP) lenses against ocular pathogens

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Objectives: To evaluate the disinfectant capacity of three commercially available disinfecting solutions and to determine if they are able to meet the FDA criteria for contact lens disinfectant solutions. To determine the effects of storage conditions on disinfectant activity because although manufacturer's recommend a 3-month period of use this does not take into account higher temperatures experienced in tropical and sub-tropical areas.
Methods: Disinfectants were challenged with *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans* according to the FDA standalone criteria. Duplicate bottles of those meeting the minimum criteria of a 3.0 log reduction in viable count (for bacteria) and 1 log reduction (for Candida) within the minimum recommended disinfection period were then stored at a range of temperatures and conditions and challenged at weekly intervals for 1 month.
Results: Boston Simplicity met the FDA acceptance criteria for the standalone procedure for all organisms and for all conditions tested. Boston Advance though meeting the criteria for all organisms when careful handling was employed, failed to adequately reduce numbers of *S. aureus* when poor handling technique over a 1-month period was utilised. Poor handling reduced activity against *Ps aeruginosa* for all solutions. Alcon Optisoak failed to meet the FDA criteria against *S. aureus* and *E. coli* in initial testing. Fridge storage resulted in reduced activity of Boston Simplicity against two organisms. All solutions showed some loss of activity after storage in a covered water bath at 30°C (simulating bathroom conditions in warm climates) for 1 month.
Conclusions: Microbial keratitis, which can lead to severe loss of visual acuity, is associated with breeches in correct handling of contact lenses. Poor handling techniques may result in loss of disinfectant activity, confirming the need for reinforcement of education of contact lens users. Storage at temperatures other than room temperature adversely affects the activity of disinfectants and clients should be advised not to store solutions in the fridge and to dispose of solutions after a shorter period of time if the ambient temperature exceeds 25°C. Failure of one solution to meet the FDA requirements may be a cause for concern.

P1176 National electronic Library of Infection in the UK – can it change clinical practice?

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Motivations: Recent technical advances resulting in a boom in medical digital libraries that have resulted in an overwhelming amount of medical information available on the Internet. However, the Internet can only play this essential role in healthcare if the knowledge provided over this powerful media is made accessible and delivered to healthcare professionals in the right form to meets their needs. Healthcare professionals often cannot find the information they need when they need it and if they do the quality may be uncertain.
National electronic Library of Infection: NeLI http://www.neli.org.uk is providing the best available evidence around investigation, treatment, management and control of infectious diseases. NeLI, a specialist library of the National electronic Library of Health (NeLH), is a digital library providing the best available

evidence-based knowledge, enhanced with medical quality tags provided by members of major professional societies and expert committees in the area of communicable disease in the UK. The quality tag consists of a bottomline critically summarising the paper, and a checklist, which answers brief questions about the methodological issues, level of evidence, potential biases and applicability of the results. The resultant quality tag and a signature of the particular society are attached to the document.

The Web log evaluation of NeLI: A web log analysis investigating data for the period from January 2002 till June 2003 was performed by Gemma Madle (g.c.madle@city.ac.uk). The results revealed that the number of users has significantly increased from 422 in January 2002 to 1154 in June 2003, the number of sessions increased from 622 in January 2002 to 1609 in June 2003. Over two-thirds of users (70%) are using IPs registered in the UK or US. Hospital-based users are spending more time in a session and are returning to visit the site more often in a month than other users. The average time spent by hospital-based users in a session was 8 min and 23 s compared with 4 min 52 s for all users and hospital-based users visit the NeLI an average of 6.71 times per month compared with 1.52 times for all users. Top ten topics pages include: Meningitis (2.82%), HIV (2.72%), Tuberculosis (2.68%), Tinea (2.55%), Antimicrobial Resistance and Healthcare Associated Infection (1.93%), *Staphylococcus aureus* (1.85%), Chlamydia (1.83%), Parvovirus (1.73%), Helicobacter (1.69%), Salmonella (91.68%).

P1177 Psychological aspects of needle-stick injury to health care workers in a teaching hospital

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Purpose: Health care workers (HCW) are exposed to daily risk of injury by needle-sticks or other medical instruments. The statistics of needle-stick injury are distributed annually and the victims are medically controlled appropriately. But, the psychological impacts on the victims of needle-stick injury have not been studied yet. We tried to evaluate the stress, anxiety levels, and depressive symptoms of HCW with the history of needle-stick injury.

Methods: Researchers consisted of psychiatrist, infection specialist doctor, and infection control nurse. They provided questionnaire items about needle-stick injury and preventive activities. Psychological scales applied in this study were Beck depression inventory; BDI, Perceived Stress Scale; PSS, and Hamilton Anxiety Scale; HAM-A. The responses from 370 HCW were analysed statistically using SPSS 10.0.

Results: The proportion of male to female was 21.6% ($n = 80$) and 78.4% ($n = 290$). Among the subjects, 23.8% ($n = 88$) were doctors, 63.0% ($n = 233$) were regular nurses, 8.1% ($n = 30$) were ward assistants, 4.3% ($n = 16$) were technicians, and 0.8% ($n = 3$) were others. The proportion of HCW with or without needle-stick injury was 71.1% ($n = 263$) and 28.9% ($n = 107$). Total PSS score was 19.48 ± 3.52, total HAM-A score was 11.39 ± 8.74, and total BDI score was 30.35 ± 6.99 at ordinary times. In case of women, the scores of all three measurement levels were higher than that in men. HAM-A and BDI scores were significantly higher among HCWs with needle-stick injury history ($P = 0.00$). PSS and BDI scores of HCW with needle-stick injury experience were higher after the occurrence of needle-stick injury than that at ordinary times. The existence of HBs Ab of HCWs with needle-stick injury history was not significant in PSS, HAM-A, and BDI scores ($P > 0.05$).

Conclusions: Women who work in general hospital reported higher level of stress, anxiety, depression compared with men. Subjects with experiences of needle-stick injury showed significantly higher level of anxiety and depression. Among the subjects with needle-stick injury experiences, higher level of depression and stress scales were observed.

P1178 Implementation of the ISO 9001:2000 standard in the Infectious Diseases Surveillance and Control Office of the Department of Public Health, Western Health Board, Ireland

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Objectives: The Infectious Diseases Surveillance and Control Office (IDSCO) is part of the Department of Public Health, in Merlin Park. The primary functions of the office are: (1) surveillance of infectious diseases for the Western Health Board; (2) production of the monthly Westfile bulletin; (3) management and control of outbreaks of infectious diseases. The need for health services to focus on quality was clearly stated in the 2001 Health Strategy for Ireland. This prompted the IDSCO team to apply the ISO 9001:2000 standard to the surveillance and control of infectious diseases in the Western Health Board.

Methods: Conforming to the ISO 9001:2000 standard involved carefully delineating the functions of the office and determining measuring points where performance could be assessed. Some steps proved problematic and the progress towards registration will be outlined, along with some of the difficulties encountered.

Results: Implementation of the ISO 9001:2000 standard and successful registration usually takes a full year. The IDSCO was registered successfully by the National Standards Authority of Ireland (NSAI) after only 10 months. This is all the more remarkable since it was the first time this standard has been applied to the surveillance and control of infectious diseases in Ireland; and may also be the first such registration in Europe. Implementation of the ISO 9001:2000 standard has resulted in the following improvements to the surveillance and control of infectious diseases in the Western Health Board: (1) The monthly surveillance bulletin 'Westfile' has been improved by the addition of formal customer feedback measurement. (2) The accuracy of regional infectious disease figures reported in the bulletin has been improved by modification of the process used to extract the data. (3) The control and management of outbreaks has been improved by addition of specific measuring points that can assess how efficiently the process is controlled at each point. (4) Continuous review, as an integral part of the ISO 9001:2000 standard; ensures that the system currently in place will be reviewed on an ongoing basis to ensure it delivers the best service possible.

Conclusion: The implementation of the ISO 9001:2000 standard in the IDSCO was a tortuous but ultimately worthwhile process. As it was a national, and possibly a European first, the experience we have gained from the successful implementation of the ISO 9001:2000 standard may assist others considering registration.

P1179 The User Satisfaction Survey – an instrument to adjust and improve clinical microbiology services

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Objectives and background: Budget cuts are general conditions of our public health care system and constitute a daily challenge. Accordingly laboratory administrators must constantly adjust laboratory services in a manner as professionally safe as possible. In connection with a major budget cut we decided to improve our basis for decision-making and involve our customers by performing a user satisfaction survey.

Study design – a survey: A questionnaire with 35 questions and 8 sections: requisition, reporting, diagnostic service, staff service, consultative service, duty/opening hours, improvements and adjustments, general comments. Participants: The questionnaire was sent to 211 employees at four different hospitals representing the following departments: paediatric, internal medicine, ICU, and surgery. Questions were constructed as postulates with six response alternatives: strongly agree, agree, both agree and disagree, strongly disagree, disagree, neither agree nor disagree.

Results (selected): Response rate, 65%. Overall, our customers were satisfied with our requisition system and our result report-

ing. Concerning our diagnostic service 32% thought that result-processing times were too long, and 20% were dissatisfied with having to phone the lab to get the report quicker. Most were satisfied with the personal service of the staff and the telephone service in connection with findings of acute clinical importance. As regards the consultative service more internal conferences focusing on particular patients and/or subjects were in demand as well as further training in microbiology and related subjects. 35% thought that knowledge of test price would limit the number of samples taken, 46% thought that better training would have a similar consequence, and 25% wanted to know more about indications for sampling.
Conclusion: The user satisfaction survey has in our hands been a profitable instrument to adjust and make our services better. From now on we will try to improve our economy and the quality of the specimens by focusing on training in microbiology, on the indications for taking samples, as well as making our customers more price-minded.

P1180 Communication methods used for implementing antibiotic policy: a pilot project in Belgian hospitals

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Objective: In 2002–2003, a pilot project funded the establishment of antibiotic management teams (AMT) and antibiotic managers (AM) in Belgian hospitals. First year activity reports were reviewed to assess the communication methods used for implementing antibiotic policies.
Methods: The pilot hospitals ($n = 35$) had a median number of 654 (from 154 to 1597) beds; 17 were general hospitals, 10 general hospitals with teaching beds and 8 teaching hospitals. Communication methods used for dissemination of antibiotic recommendations within the institution were categorised; 1, 2 and 3 points were granted to passive, active and personalised methods, respectively, and the cumulative sum was used as a score for ranking hospitals.
Results: Communication actors were identified as the AM (sender), guidelines (message) and healthcare staff (receiver). In passive methods, only the sender knows that the message was emitted. These included: mailing (33), intranet (6), internal newsletter (9), e-mailing (2) and posters (1). In active methods, the sender knows that the message was emitted and at least received. These included: information meetings (lectures, courses, staff meetings) (13) and infection ward rounds (11). In personalised methods, the sender knows that the message was emitted, received and understood. These included: individual feedback (6), face to face meeting with AM (18), audit (2) and computer-assisted prescribing (9). Hospitals used an average of 3.6 communication methods (1–8). The mean communication score was of 6.5 points (1–15). General hospitals, general hospitals with teaching structure and teaching hospitals used an average of 3.2, 5 and 3.7 methods with a mean score of 5.8, 6.3 and 8.3, respectively. No difference was seen by hospital size in the number of methods used or communication score.
Conclusion: Advertisement type categorisation of communication methods showed that all hospitals used at least one passive method, 39% used at least one active method and 55% used at least one personalised method. The quality of communication was higher in hospitals with teaching affiliation ($P = 0.05$).

Antibiotic use and surveillance

P1181 Reduction in outpatient antibiotic sales to pre-school children – interrupted time-series analysis of weekly antibiotic sales data in Sweden, 1992–2002

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Objectives: The availability of extensive retrospective data on drug sales in Sweden provides unique opportunities to detailed studies of trends in antibiotic use. We analysed the weekly sales of antibiotics prescribed in outpatient care to children aged 0–6 years between 1992 and 2002, to identify trends in sales during the period.
Methods: An interrupted time series model controlling for seasonality was used to examine the datasets for significant level and trend shifts, with correction for autocorrelation. The total sales of antibiotics (ATC group J01), as well as the individual subgroups commonly used to treat respiratory tract infections in children were studied: phenoxymethyl-penicillin (J01CE); extended-spectrum penicillin (penicillins with extended spectrum and combination of penicillins including beta-lactamase inhibitors: ATC groups J01CA and J01 CR) and macrolides (ATC group J01FA). Data were expressed as number of prescriptions/1000 inhabitants/week.
Results: Overall, sales have decreased in all studied groups during the period. For the total antibiotic sales, two significant trend breaks in 1996 and 1999 could be identified. The first and the third period showed a declining trend in the number of dispensed prescriptions, while the period between 1996 and 1999 in opposite showed an increasing trend. Trends in extended-spectrum penicillin sales followed the same pattern, with two trend breaks at 1995 and 1998. As in the total antibiotic group, the baseline and the third segment show decreasing trends, while the trend was increasing in the middle segment. For phenoxymethyl-penicillin, no significant trend or level shift could be identified, and the trend was slowly decreasing during the whole observation period. In macrolide sales, a steep decrease was seen in the baseline segment, but after a trend break in 1995 the trend in sales has been constant.
Conclusions: The weekly data material used for the analysis is unique with regard to population coverage and data quality. Even though no causal relationship can be established between trend breaks and isolated interventions introduced during the study period, it is obvious that the work for a more prudent outpatient antibiotic use in children in Sweden has been successful. However, the reasons behind the increasing trend in broad-spectrum antibiotics seen in the middle of the decennium needs to be explained.

P1182 Blood cultures as a surrogate marker of case-mix for adjustment of hospital antibiotic consumption

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Objectives: Surveillance of hospital antibiotic consumption is an important step towards appropriate use. Consumption is generally adjusted to an indicator of hospital occupancy, e.g. patient-days. Such indicators are readily available, but they do not allow adjustment to the case-mix. We investigated whether the number of blood samplings drawn for culture could be used as a surrogate marker of the burden of infection in a hospital ward, and therefore could serve for adjustment of antibiotic consumption to case-mix.

Methods: We first determined one set of clinical and laboratory criteria to define opportunities for prescription of antibiotics (OPA), based on retrospective review of adult patients hospitalised in one university hospital (derivation sample: 178 patients; validation sample: 200 patients). The correlation between OPA and actual antibiotic prescription was good (positive predictive value (PPV) 81%, negative predictive value (NPV) 95%). Then correlation between blood cultures and OPA was established for a medical ward. Finally, we compared antibiotic consumption in this ward measuring it either in defined daily doses (DDD) per patients-day, or in DDD per blood culture, for 16 consecutive trimesters.
Results: Blood cultures had a 70% PPV and a 97% NPV of predicting OPA. Of the 16 consecutive trimesters, 2 were found showing up high antibiotic consumption (>95% CI for the mean) as measured in DDD either per patients-day or per blood culture. For three trimesters antibiotic consumption was high according to one measurement method only. Analysis of random samples of 50 patients per trimester showed that this discrepancy was not due to the instability of blood cultures as marker of OPA. It also confirmed that blood cultures were more accurate for adjustment of consumption since (i) for one trimester with high consumption in DDD per patients-day only, this result was actually due to higher incidence of OPA; (ii) two trimesters with high consumption in DDD per blood culture only had indeed low incidences of OPA, and therefore a probable problem with antibiotic use that was not identified when measured in DDD per patient-day.
Conclusion: Blood cultures are a stable marker of OPA in a medical ward. This allows the identification of periods deserving a detailed investigation given the unexplained high antibiotic consumption.

P1183 Audit of the usage of broad-spectrum antibiotics in a Belgian university hospital: much room for improvement!

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Objectives: In order to improve the quality of antimicrobial prescription local written guidelines for empiric and documented antibiotic therapy were developed and launched in April 2002. A specific prescription formulary aiming to evaluate the usage of broad-spectrum antibiotics was introduced in June 2003.
Methods: From 1 June to 31 August 2003, we assessed the prescription of all broad-spectrum antibiotics. Parameters recorded and analysed included patient's data (clinical diagnosis, microbiological data) and antibiotic's specific data (choice of indication, daily dose, mode of administration and duration of therapy. Adequacy of therapy was defined according to our local reference guidelines.
Results: The patient cohort consisted of 266 patients presenting 301 infectious episodes (210 in patients from various medical units and 91 from patients in different surgical wards). Four units (haematology, pneumology, cardiovascular and digestive surgery) accounted for almost 60% of all prescribed antibiotics. Treatments were considered appropriate in 221 (73.7%) episodes and non-adequate in 80 other episodes (including 29 indeterminate therapies due to lack of microbiological/clinical data). Adequate therapies were recorded in 31/34 epidoses with concomitant bacteraemia. The agents most frequently inadequately prescribed included the fluoroquinolones (FQs) (41 [52%]), the aminoglycosides (16 [18%]) and piperacillin/tazobactam (11 [14%]). For the FQs, the main reason accounting for inadequate prescription was the lack of indication (33/41), for aminoglycosides excessive duration was found in 13/16 episodes. Inadequate therapies were predominantly observed in empiric therapy (59/80). The types of infections most frequently concerned in inappropriate therapies were: urinary tract (UTI) (30%), abdominal (29%) and respiratory tract infections (22%). The units in which inappropriate prescription patterns were most frequently observed included digestive surgery (38%), gastroenterology (47%) and neurology (25%).
Conclusion: Overall this audit highlighted areas (types of infections and specialty-specific) where discordances of prescribing with the empiric or documented antimicrobial guidelines were frequently observed. Of most concern was the frequent inappropriate usage of FQs as well as the inadequate diagnosis/management of UTI. This study further highlights the need for the establishment of continued educational programs focusing on appropriate antimicrobial prescribing.

P1184 Development of a paediatric daily defined dose system for the measurement of antibiotic consumption in paediatric units

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Background and objectives: Antimicrobial consumption is frequently measured using the daily defined dose (DDD) system assigned by WHO to every antimicrobial drug. DDD is the presumptive average maintenance daily dose of a drug used for its main indication in adults. The fact that the existing DDDs are derived from adult doses could compromise the validity of this tool in studies involving paediatric patients. Since drug doses in children are most frequently based on body weight (BW), the calculation of paediatric DDDs should take into account the mean BW of children admitted to a paediatric unit. Our objective was to develop a method for the calculation of paediatric and neonatal DDDs, for common antimicrobial drugs used in a tertiary hospital.
Methods: We calculated the mean BW of 229 children consecutively admitted to the paediatric wards of our hospital. We then calculated the paediatric DDD for a given antimicrobial with the equation: ped DDD = mean BW (kg) × dose (mg/kg), where dose (mg/kg) is the average or usual recommended paediatric dose of the drug, according to approved textbooks or formularies. For neonatal DDDs, we multiplied the mean BW of 255 infants consecutively admitted to the neonatal unit [(birth weight + discharge weight) / 2] with the average or usual recommended neonatal dose of the drug. We used ceftriaxone to apply our proposed paediatric and neonatal DDD system.
Results: The mean BW of the paediatric patients was 18.42 kg (95% CI: 16.48–20.35 kg) and that of the hospitalised neonates was 2.57 kg (95% CI: 2.48–2.66 kg). Based on our method, with an average paediatric dose of 75 mg/kg and a neonatal dose of 50 mg/kg of ceftriaxone, the paediatric DDD was found to be 1.4 g and the neonatal DDD 0.13 g. By employing the DDD assigned to ceftriaxone by WHO (2 g), the consumption of this drug during 2002 in three different departments of our hospital (adult, paediatric, neonatal) was 5.11, 2.87 and 1.09 DDDs per 100 bed-days, respectively. By using, however, the adjusted DDD system for the paediatric and neonatal wards, the consumption was found to be 5.11, 4.11 and 16.83 DDDs per 100 bed-days, respectively.
Conclusion: The development of paediatric and neonatal DDDs with this BW-based approach, using collective data from different hospital departments and countries, could greatly facilitate antibiotic consumption studies involving paediatric or neonatal units.

P1185 The impact of multiresistance in risk factor analyses of antibiotic exposure among antimicrobial-resistant bacteria

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Background: Antibiotic resistant infection is a growing medical concern. Therefore, accuracy of epidemiological studies is fundamental to elaborate the best interventions to reduce the spread of antibiotic resistance. Previous risk factors analyses have assumed single-drug resistance in the bacteria under study. Since many bacteria may be multidrug-resistant, we hypothesise that the assessment of antibiotic exposure as risk factor for the emergence

of antibiotic resistance will differ depending on whether single-drug or multi-drug resistance is analysed.
Methods: A case–case–control study was performed to determine differences in intravenous antibiotic use in the previous 30 days among two groups of cases. The first group of cases included patients harbouring *Pseudomonas aeruginosa* (PA) resistant only to ciprofloxacin (CR-PA) and the second group of cases included patients harbouring CR-PA, resistant to one or more of the following antibiotics (MDR-PA): ceftazidime (CFZ), aminoglycoside (AMG), imipenem (IMP) or piperacillin-tazobactam (PipT). Controls were selected among patients admitted to same hospital not harbouring PA. Matching for number of days from admission to CR-PA or MDR-PA recovery among cases and duration of hospitalisation among controls was performed. Potential confounders were included in a conditional logistic regression analysis.
Results: A total of 384 patients had been enrolled. Forty-two patients had CR-PA and 151 MDR-PA recovered in two University hospitals were compared with 192 controls. Use of quinolones was independently associated with CR-PA (OR 2.1, 95% CI 1.3–3.2, $P < 0.01$) and MDR-PA (OR 2.2, 95% CI 1.2–3.8, $P < 0.01$), while use of 3rd gen. cephalosporins (OR 2.3, 95% CI 1.4–4.1, $P < 0.01$), AMG (OR 2, 95% CI 1.1–3.9, $P = 0.01$), IMP (OR 2.4, 95% CI 1.1–5.3, $P = 0.02$), and PipT (OR 2.5, 95% CI 1.1–6.6, $P = 0.02$) were significantly associated to MDR-PA only. There were no statistically significant differences between CR-PA and MDR-PA with controls for age, comorbidities, intensive care unit or health-care facility exposure, and duration of therapy within 30 days.
Conclusion: When multidrug resistance is taken into consideration, significant differences in antibiotic exposures are identified between single-drug resistant vs. multidrug-resistant bacteria. Future epidemiological studies should consider co- and cross-resistance to other antibiotics when analysing risk factors for the emergence of antimicrobial resistant bacteria.

P1186 Large-scale nationwide point prevalence study of indications for antibiotic use in 54 Swedish hospitals in 2003

G. Skoog, O. Cars, K. Skärlund, S. Elowson, H. Hanberger, I. Odenholt, M. Prag, J. Struwe, E. Torell, P. Ulleryd, M. Erntell – The STRAMA-program

Objectives: The objective of the study was to introduce a nationwide survey system for frequent assessment of the use of antimicrobial agents in relation to diagnose. The STRAMA-groups have performed the first point prevalence study, PPS, using a web-based reporting system.
Method: A nationwide PPS with one personal visit to each department was performed within a 2-week period in November 2003. The protocol was designed to present demographic data as well as the amounts and indications for antimicrobial agents against bacteria and fungi. Treatments were recorded in relation to diagnoses and prophylactic use, community acquired (CAI) and hospital acquired infection (HAI). Nineteen pre-defined diagnosis groups were used.
Results: 54 hospitals participated in the study. 4178 patients treated with antimicrobial agents were included out of 13 529 admitted to nine university hospitals (1538 treated patients), 20 county hospitals (1855 patients), and 25 local hospitals (785 patients). 31% of the admitted patients were treated with antimicrobials. A total of 4395 treatments were recorded. 266 (6.4%) were given to children (<17 years) and 49.9% to women. The indication for treatment was CAI in 17%, HAI in 9% and prophylaxis in 6%. For adults cultures were taken before oral treatment in 60% and before parenteral treatment in 69%. The most commonly used antimicrobials for adults, expressed in DDD, in treatment and in prophylaxis were cephalosporins (23 and 18%), isoxazolyl-pc (13 and 47%), fluoroquinolones (12 and 9%), broadspectrum-pc (10 and 4%). The total amount of antimicrobials used for adults was 40.3 DDD/100 admitted patients. For children the corresponding results in number of treatments were; cephalosporins (42 and 31%), beta-lactamase sensitive penicillins (8 and 8%), tienamycins (8 and 1%), glycopeptides (7 and 0%), and co-trimoxazole (6 and 15%). The number of treatments was 39.9/100 admitted children. Analysis of different diagnoses shows over-use of cephalosporins in community acquired pneumonia and fluoroquinolones in urinary tract infections.
Conclusions: The PPS method was successfully introduced resulting in one of the largest surveys in Europe of antimicrobial hospital treatment. The study describes suboptimal prescription patterns for certain diagnoses.

P1187 Antibiotic consumption in ambulatory care in Germany: a regional NUTS-I level analysis

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Objectives: The MABUSE (Medical AntiBiotic Use Surveillance & Evaluation) network, a German cooperative group of infectious disease specialists, WIdO, the SARI programme and hospital pharmacists, collects antibiotic consumption data in ambulatory (AC) and hospital care (HC) settings. In the present study we evaluated geographical aggregation of AC data on the level of NUTS-I (Nomenclature of Units for EU Territorial Statistics) areas in order to allow regional analysis of consumption data which may be more meaningful than presenting national estimates for a large country with cultural, historical and geographical diversity and long borders with other EU countries.
Methods: AC data (covering all antibiotic prescriptions within the compulsory health insurance, i.e. 86% of the total population, years 2001 and 2002) were calculated using the WHO/ATC DDD definitions and expressed as DDD/1000 per year for the 16 German NUTS-I areas (corresponding to federal states). In a second analysis, the four small-size NUTS-I areas Bremen, Hamburg, Berlin and Saarland were included into 12 geographically fitting larger areas in order to have a more balanced data presentation in terms of geographical and structural organisation.
Results and conclusions: AC consumption in 2001 ranged between 3322 and 5802 DDD/1000, corresponding to 9.1 and 15.9 DDD per 1000 population and day). There was no major change between 2001 and 2002 values, except that non-small-spectrum beta-lactams slightly increased. NUTS-I areas in the northern and western parts of the country showed in 2001 much higher consumption (range, 5286–5802 DDD/1000) than those in the southern part (range, 4389–4432 DDD/1000) or in the east (range, 3322–4391 DDD/1000). This pattern did not change after our modified NUTS-I analysis: northern part, 5286–5734 DDD/1000, western part, 5006–5601 DDD/1000, southern part, 4389–4432 DDD/1000, eastern part, 3769–4232, respectively. This regional representation of German consumption data allowed useful evaluation of patterns of different antibiotic drug class and subgroup consumption and may also be valuable for interregional comparisons within the ESAC project.

P1188 SARI – Surveillance of Antibiotic Use and Bacterial Resistance in German Intensive Care Units

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Objective: To provide epidemiological data on antibiotic resistance, prophylactic or therapeutic use of antibiotics and on the correlation between antibiotic use and resistance rates in intensive care units (ICUs) in Germany, which are considered to be high risk areas for the emergence and spread of multi-resistant bacterial pathogens.
Methods: ICUs collected data on patient days, antibiotic use (defined daily doses= DDD according WHO) and resistance rates of selected antimicrobial pathogens on all non-duplicate clinical isolates. Antibiotic use density (AD) is calculated in DDDs/1000 patient days. The data were recorded, analysed centrally and communicated to the participants every 3 months.

Results: Project SARI started in February 2000, and includes data on antibiotic use and resistance rates in 38 medical, surgical and interdisciplinary ICUs. To date (from February 2000 to June 2003), a total of 1142 months, 413 065 patient-days and 550 288 defined daily doses (DDDs in accordance with the WHO) have been covered, with a mean antibiotic usage density (AD) of 1335 DDDs/1000 patient-days and resistance data on 37 612 isolates from ICUs. In all the ICUs, mean antibiotic usage rates are highest for penicillins with lactamase inhibitor (AD 305.8) and quinolones (AD 137.1). Cumulative data for ICUs testing in accordance with DIN ($n = 22$) show an MRSA rate of 22.7%, and a ciprofloxacin resistant *Escherichia coli* rate of 11.5%. Comparison of these resistance rates for the time periods July 2000 to June 2001 and July 2002 to June 2003 (Wilcoxon test) shows a significant increase in MRSA and ciprofloxacin resistant *E. coli*. Use of ciprofloxacin correlates significantly with the rate of methicillin-resistant *S. aureus*, as does imipenem use with the rate of imipenem resistant *P. aeruginosa*.
Conclusion: National and local data on ICUs are necessary for effective infection control. They offer a basis from which to improve infection control and antibiotic management in ICUs (http://www.sari-antibiotika.de).

P1189 Reduction of antibiotic use by a community-oriented educational programme

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Introduction: Avoiding excessive use of antibiotics is the clue for combating community acquired antibiotic resistant pathogens.
Objective: to implement and evaluate the efficacy of a community intervention program of continuous medical education oriented to primary care clinics with emphasis on appropriate use of antimicrobial drugs.
Materials and methods: From October 2000 to April 2003 we conducted a prospective educational study in community primary care clinic based on 'guidelines for antimicrobial treatment in primary care'. The aim was to promote influenza vaccination and judicious antibiotics use specially for respiratory infections. Sixteen community clinics (168 644 patients) were included: 8 = Intervention Group (IG = 86 330 patients) and eight Control Group (CG = 82 314 patients). Total population in this area (442 700 patients) was evaluated as Reference Group (RG). Total volume of antibiotics prescribed to adult patients was evaluated and measured as DDD/1000 patients. Narrow-spectrum agents (NSA) and broad-spectrum agents (BSA) were defined. Four consecutive winters: from October 1999 to February 2000 as baseline data, 2000–2001 after guidelines distribution and 2001–2002 to 2002–2003 as intervention period were evaluated.
Results: A decrease in the total use of antibiotics was measured in all the groups, but more significantly in the IG [RG:6.4%, CG:16.4% IG:20.1% ($p < 0.0001$ for all)]. Main change was noted in BSA [RG:8.2%, CG:4.9%, IG:17% DDD/1000 patients ($p < 0.0001$ for IG only)].
Conclusions: The present study showed that a community-oriented educational programme significantly reduced antibiotic use in the Interventional Group in general and in the broad-spectrum antibiotic group in particular. More efforts and amplification of this type of interventions are imperative to stop increasing antimicrobial resistance.

P1190 Evaluation of compliance to perioperative antibiotic prophylaxis guidelines

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Objectives: To evaluate surgeons' adherence to the hospital guidelines for perioperatively administered antibiotics in both planned and urgent interventions.
Methods: (1) Retrospective analysis in all planned surgical interventions performed between February and June 2002. Hospital guidelines: a standard prophylactic regimen, varying according to type of surgery, is printed on the anaesthesiology record via the hospital information system (HIS). The anaesthesiologist is expected to follow these recommendations; they can however be overruled by the surgeon. Data analysis: planned and actually performed intervention and administered antibiotics data were extracted from the HIS. (2) Prospective analysis of 40 consecutive urgent interventions performed in April 2003. Hospital guidelines: no standard regimen can be generated by the HIS in these cases; instead, a handout listing the regimen for the most frequently performed urgent interventions is available in the operating room (OR). Data analysis: A junior staff member daily collected OR-tarification/medication prescription forms and interviewed surgeons.
Results: (1) Only the 1051 cases where there was total agreement between planned and performed interventions (=75% of all planned interventions) were analysed. Overall, antibiotic prophylaxis was correct in 73% of interventions. In only 4.8% of cases where prophylaxis was indicated and given, another molecule than the one proposed, was administered. Incorrect prophylaxis was observed in 2.5% of cases where an antibiotic was given although it was not indicated and in 24.4% of cases where no antibiotic was given although it was indicated. Many of these cases were laproscopic interventions for which the surgeons – having acquired more experience with these techniques – had asked the anaesthesiologist to diverge from the original guidelines. (2) In 30 of 40 (75%) urgent operations antibiotic prophylaxis was given if indicated or withheld if not. In only 6 of 14 interventions where antibiotic prophylaxis was indicated the right molecule was administered (4) or the motivation of divergence was correctly registered (2). Many surgeons did not know about the hand-out.
Conclusions: Providing easy access to guidelines improves compliance with adequate prophylaxis: the choice of antibiotic was more adequate in planned than in urgent interventions. In addition, guidelines should be adapted regularly and attention should be given to good communication.

P1191 Use of overnight processing of bacteriology samples to modify antibiotic prescribing habits in the community

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Objectives: Through the use of accelerated bacteriology laboratory examination (ABLE) of community specimens, antibiotic prescribing in the Grampian region of Scotland has reduced by 30% in the last 6 years. Antibiotic use declined significantly more in general practices that used the system than didn't and more in Grampian compared with the rest of Scotland. In this study, we present an audit of antibiotic prescribing in Grampian and of how ABLE is being used to reduce prescribing.
Methods: Ten general practices were audited in the spring of 2003, including five high ABLE users and five low-users. The low-users of the ABLE service provided prescription lists for all consecutive patients receiving an antibiotic. The high-users of the service provided prescription lists only for consecutive patients entered into the ABLE system. Antibiotic prescription data and laboratory data were entered into an Excel spreadsheet and analysed using SPSS.
Results: Grampian is in the N.E. of Scotland with a population of 540 000 and approximately 90 general practices. A total of 699 patients were audited of which 357 were entered into the ABLE system and 342 were not. A total of 31% were male and 69% female. 419 specimens (367 ABLE, 62 non-ABLE) were sent for culture of which 162 were positive (130 ABLE, 32 non-ABLE). A total of 43.2% were urines, 27.7% throat swabs, 8.8% sputum samples and 4.5% wound swabs. An antibiotic was prescribed in 472 cases including 130 (36.3%) of the 357 ABLE patients, all of whom were culture positive. Totally, 27.6% of ABLE patients returned to the GP surgery for their prescription, 6.7% had the prescription sent to their community pharmacy and 65.7% had a

delayed action prescription. In all, 32.3% of patients made a repeat visit to their GP, largely because of persistent symptoms. The repeat visit rate was very similar between the two groups (104/324 low ABLE vs. 111/343 ABLE).
Conclusions: This audit suggests that ABLE is being used correctly, with only 33% of ABLE patients receiving an antibiotic prescription at first consult. This figure is very similar to our original study. It can be concluded that ABLE is being used correctly and that it is likely to be partially responsible for the lower antibiotic prescribing rates in Grampian.

P1192 Influence of an antibiotic facilitator in a surgical unit

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Objectives: Improved liaison with the microbiology laboratory and auditing of prescriptions have been suggested as possible ways of improving the quality of antibiotic prescribing. These strategies were investigated in a large teaching hospital where antibiotic consumption is high.
Methods: A clinical pharmacology MSc student audited prescribing on two general surgical units in our hospital. In one unit (I) she attended daily morning ward rounds and provided copies of the latest microbiology reports for discussion.
Results: 209 patients were studied over a 3-month period. Mean hospital stay was 8 days. In all 127 patients received prophylaxis (P), 97 received empiric therapy (E) and 15 received both P and E. Both units had similar rates of antibiotic prescribing; 19.7% in control (C) vs. 21.3% in I. Combination therapy was used in 55% of patients, usually metronidazole plus either cefotaxime (CTX), gentamicin or trimethoprim. Co-amoxiclav was the most common mono-therapy. Most P was single dose although 14 and 21 patients were given two and three doses of CTX, respectively. Although not statistically significant, the number receiving E (97) and microbiology tests (151) were both higher in I (54 vs. 46% and 56 vs. 44%). Negative culture results (57% of I and 37% of C) led to discontinuation of treatment in only one patient. In light of positive culture results the proportion of patients where E was satisfactory was higher in I than C [11 of 21 vs. 3 of 18 ($P = 0.06$)] although appropriate streamlining was carried out on all but one patient. Positive culture results led to initiation of therapy in three patients.
Conclusions: I used the laboratory more frequently than C and also used more appropriate E. However, the effects of the interventions were probably modest as C was quick to streamline therapy on receipt of culture results. An antibiotic facilitator had a modest but positive impact on prescribing and use of laboratory services and could be used in a wider role to delay or stop treatment more quickly in minor infections and shorten P.

P1193 Perioperative antibiotic prophylaxis – adherence to local hospital guidelines

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Objectives: The use of antimicrobial prophylaxis (AMP) for selected surgical procedures is one of the measures used to prevent the development of surgical site infections (SSIs). In our hospital, the guidelines for surgical AMP were developed in 2002 according to the CDC recommendations for the prevention of SSIs. The main recommendation was the administration of cefazolin or cefoxitin 30 min before skin incision. The aim of the current study was to evaluate the prescribing patterns of surgeons for AMP before the implementation of the guidelines and to study adherence to the guidelines after its enforcement.
Methods: Retrospective analysis by reviewing anaesthetic records within two periods each with the duration of one month: September 2001 (a year prior to the implementation of the above guidelines, period I) and September 2003 (a year after the implementation of the above guidelines, period II). The following data were recorded: type of procedure, choice of antibiotic, timing of the first dose, dosing interval of antibiotic and whether the AMP was indicated or not.
Results: There were 1238 and 1404 operations performed within period I and period II, respectively. AMP was applied in 450 (36%) of cases in period I and 732 (52%) of cases in period II. Antibiotics used in period I: ceftriaxone 22%; ampicillin combined with gentamicin 19%; cefuroxime 17%; cefazolin 9%. Antibiotics used in period II: cefazolin 84%; cefoxitin 7%. Timing of the first dose was appropriate in 32% (period I) and 45% (period II) of cases. Antibiotic indication, choice, dosing interval were concordant with the hospital guidelines in 80, 89, 96%, respectively, in period II. Overall percentage of adherence to all studied aspects of the guideline was 57%.
Conclusions: This study shows that the implementation of the guidelines influences the prescribing patterns of surgeons for AMP. Perioperative use of antibiotics increased within period II. Timing of the first dose of AMP needs to be improved in particular. Further educational efforts are considered necessary in the future.

P1194 Increase and change in patterns of hospital antimicrobial use, Denmark, 1997–2001

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Objectives: To analyse the changes in antimicrobial use in Danish public hospitals over the period 1997–2001.
Methods: Data on the number of WHO defined daily doses (DDD) of antimicrobials were obtained from the Danish Medicines Agency. Data on the number of patient-days were obtained from the National Board of Health. Psychiatric hospitals, a neurological centre, a rehabilitation centre, as well as psychiatric, outpatient and day wards were excluded. For each hospital, we calculated the number of DDD per 100 patient-days for both the total antibacterials for systemic use, i.e. group J01 of the Anatomical Therapeutic Chemical (ATC) and for selected classes of this group.
Results: Between 1997 and 2001, antimicrobial use in Danish hospitals increased 18% from 38.7 to 45.7 DDD per 100 patient-days. Most of the increase (62%) was attributed to commonly used groups of antimicrobials, mainly penicillins with extended spectrum (ATC group J01CA) and the beta-lactamase sensitive (J01CE) and resistant penicillins (J01CF) (Figure 1). There was also an increase in newer 'broad-spectrum' antimicrobials, i.e. combinations of penicillins with beta-lactamase inhibitor (J01CR), cephalosporins (J01DA), carbapenems (J01DH) and fluoroquinolones (Figure 1), contributing to 38% of the increase in total use. These 'broad-spectrum' antimicrobials represented 19% of total use in the Danish hospital sector in 2001. Moreover, there were large variations among hospitals in their use of both the total antimicrobials and the specific classes.

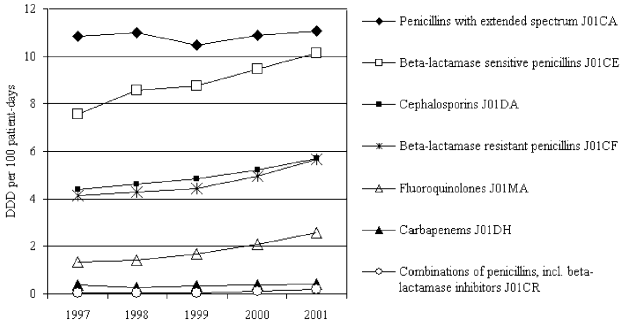

Figure 1. Use of selected antimicrobials in hospitals in Denmark, 1997–2001.

Conclusions: This increase and change in pattern of antimicrobials used in Danish hospitals is a cause of concern and necessitates close monitoring. Possible explanations include (1) a trend towards earlier discharge resulting in a higher number of admissions (possibly more doses for surgical prophylaxis, (2) longer hospital stays required mostly by sicker patients who more often receive antimicrobial treatment, (3) the more frequent prescription of combination therapy, and (4) intensive marketing of 'broad-spectrum' antimicrobials. As a first intervention, we are now establishing stratification criteria and standards for comparisons of antimicrobial use levels among hospitals with similar characteristics.

P1195 Restricted antibiotic evaluation and pharmacoeconomic outcomes

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Objectives: Implantation of a program to evaluate the quality of restricted antimicrobial agents prescription and pharmacoeconomic outcomes of the intervention proposed by the multidisciplinary antimicrobial management team (AMT).
Methods: AMT is integrated by an infectious disease physician, a clinical pharmacist and a clinical microbiologist. From 1 October to 23 November 2003, all hospitalised patients with restricted antibiotic prescription were daily reviewed by the AMT. Revision criteria were according to the Infection Disease Committee Guidelines, based on the Sanford Guide to Antimicrobial Therapy. Clinical, microbiological and pharmacological data were collected from each patient. Antibiotic therapy appropriateness, restricted therapy eligibility, length treatment and economics outcomes were evaluated, and an alternative antibiotic was considered when necessary. Economic evaluation included only the medication cost. Statistical analysis was performed with the SPSS package. Non-parametric test were used.
Results: 70 patient prescriptions were reviewed. Restricted agents prescribed were ceftazidime (34.3%), imipenem (25.7%), amikacin (17.1%), piperacilin-tazobactam (10%), cefepime (7.1%), meropenem (2.9%), aztreonam (1.4%) and teicoplanin (1.4%). Most frequent diagnostic was sepsis/febrile syndrome (32.9%), following by gastrointestinal (20%) and respiratory (17%) pathologies. Microbiological culture was performed in 64.3% of patients. Antibiotic therapy was not recommended in 15.7% cases and the restricted antibiotherapy were not suitable in 54.3%. The average of treatment length prescribed was 7 days, which is statistically different respect to the median 4 days recommended by the AMT ($P < 0.001$). These extra days would result in a 9460€ cost. The total restricted antimicrobial cost was 17067€ (243€/patient), where 1675€ correspond to not appropriate therapy amount. Approximately the half amount of right antibiotic prescription belongs to not eligible restricted therapy (7467€), that could be substituted by better alternatives (1950€). The total AMT intervention saving would have been 7190€ (102€/patient).
Conclusion: Restricted antibiotics prescription is not appropriate in more than half the cases evaluated. There is a low microbiological justification of prescription and the antibiotic treatment length is overestimated. The AMT intervention would result in an important saving.

P1196 Comparison of antimicrobial resistance in hospital-acquired and community-acquired bacteraemia

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Objective: To compare prevalence of antimicrobial resistance in blood-derived isolates from patients hospitalised for more than 48 h (in >48), assumed to represent hospital-acquired infections, with those from all other patients (in <48).

Methods: Bacteraemia isolates were collected from a total of 29 laboratories in the UK and Ireland in 2001 and 2002, excluding duplicates within one week. Isolates were centrally tested by BSAC agar dilution MIC method and categorised by BSAC breakpoints. Percentages were compared by exact test.
Results: The table shows percent resistant (intermediate for penicillin, positive for ESBL). Estimates of percentage resistance were higher in isolates from patients hospitalised >48 h for most antimicrobials in all six groups reported here. The increase was statistically significant for most antimicrobials with *S. aureus*, reflecting the endemic presence of EMRSA 15 and 16 in our hospitals. It was also significant for many antimicrobials with CNS; penicillin with *S. pneumoniae*; piperacillin-tazobactam, cefuroxime and ESBLs with klebsiella; and cefuroxime and ESBLs with enterobacter. In other cases, the study lacked power to detect increases from a low baseline: even 200 isolates per group are too few to detect a change from 1 to 5%, 5 to 10%, or 20 to 30% reliably at 5% significance level. There was no resistance to linezolid in these Gram-positive bacteria, or to imipenem in these Enterobacteriaceae.

	S. aureus		CNS		*S. pneumoniae*	
	in<48 $n = 182$	in>48 $n = 167$	in<48 $n = 111$	in>48 $n = 241$	in<48 $n = 324$	in>48 $n = 96$
Oxacillin-R	29.1	50.2*	64.9	79.3*		
Penicillin-I & R					6.2	13.5*
Ciprofloxacin-R	33.5	48.3*	37.8	56.8*	24.4	20.8
Erythromycin-R	33.0	48.3*	56.8	66.8	17.3	16.7
Tetracycline-R	3.8	2.6	48.6	56.0	4.0	3.1
Gentamicin-R	1.6	9.0*	45.0	66.8*	100.0	100.0
Imipenem-R	8.2	18.4*	10.8	20.3*	0.0	0.0
Piperacillin/ tazobactam-R	29.7	49.4*	24.3	34.0	6.5	10.4
Linezolid-R	0.0	0.0	0.0	0.0	0.0	0.0
	E. coli		*Klebsiella* spp.		*Enterobacter* spp.	
	in<48 $n = 296$	in>48 $n = 177$	in<48 $n = 198$	in>48 $n = 236$	in<48 $n = 99$	in>48 $n = 231$
Amoxicillin-R	57.8	62.1	98.5	98.7	94.9	95.2
Amoxicillin/ clavulanate-R	23.3	25.4	7.6	11.9	90.9	92.2
Cefuroxime-R	8.1	13.0	14.1	22.0*	62.6	73.6*
Ciprofloxacin-R	7.1	7.9	6.1	8.5	9.1	11.3
Gentamicin-R	11.8	10.7	4.0	8.5	10.1	15.2
Imipenem-R	0.0	0.0	0.0	0.0	0.0	0.0
Piperacillin/ tazobactam-R	3.0	4.5	5.6	11.4*	15.2	23.8
ESBL-positive	1.0	2.3	2.5	7.2*	2.0	10.0*

CNS = coagulase-negative staphylococci. ESBL = extended spectrum beta-lactamase
R = resistant, I = intermediate. *$P < 0.05$

Conclusion: Antimicrobial resistance was more prevalent in hospital-acquired infection than in infections acquired elsewhere. ESBLs were much more prevalent in, but not exclusive to, isolates from patients hospitalised >48 h.

P1197 The impact of a nationwide antibiotic restriction policy on antibiotic usage and cost

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Objective: Antibiotics are the most commonly prescribed drugs in Turkey. Most of the health expenditure is spent for antibiotics. The government has released a regulation in February 2003 in order to decrease antibiotic expenses, and injudicious use of antimicrobial agents. The aim of the study is to assess the impact of

this nationwide antibiotic restriction policy (NARP) at a university hospital.
Materials and methods: Ibn-i Sina Hospital is a 1200-bed university hospital in which there was not any restriction on antibiotic prescription before February 2003. The regulation released by government in February 2003, divided the antimicrobial agents into four groups. The first group contains the antibiotics that can be prescribed by any physician. Second group antibiotics can be prescribed by any specialist. Third group antibiotics can be prescribed by specialists for 3-day use but infectious diseases (ID) specialist approval is necessary for longer treatments. Last group antibiotics can be prescribed by only ID specialists. All hospitalised patients were visited on 18 February (prior to NARP) and on 15 September 2003 (after NARP), by ID specialists. Data were recorded on individual forms for each patient receiving antimicrobial agent. The appropriateness of antimicrobial treatments were assessed according to published guidelines by two ID specialists and one ID professor. Chi-square test was performed, a P value of <0.05 was accepted as significant.
Results: Of the 856 hospitalised patients, 178 (20.7%) were receiving antimicrobial treatment on the 1st prevalence day (18 February). On the 2nd prevalence day (15 September), 179 (20.8%) of 857 hospitalised patients were on antimicrobial treatment. Before and after NARP, 64 and 71.5% of antimicrobial treatments were judged appropriate, respectively ($P = 0.131$). Inappropriate antibiotic usage was higher in prophylactic use than empiric use (40 vs. 10%, $P < 0.01$). The main reason for inappropriateness in prophylactic use was longer duration of prophylaxis than needed. Consultation requests from medical departments increased from 8 to 36% ($P < 0.01$). After NARP 42.4% of the empirical treatments was begun after ID consultation, while it was 14.2% ($P < 0.01$) before NARP. The daily cost of inappropriate use was 2.615 and 2.142 dollars, before and after NARP, respectively.
Conclusion: NARP had a good but unsatisfactory impact on antibiotic usage and cost. We need other interventions for an optimal outcome.

P1198 The relation between *Escherichia coli* resistance and antibiotic consumption in Europe: an EARSS/ESAC study

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Objectives: EARSS (European Antimicrobial Resistance Surveillance System) and ESAC (European Surveillance of Antibiotic Consumption) explored the linkage of their databases to investigate the relationship between resistance of invasive *Escherichia coli* isolates and antibiotic consumption in ambulatory care in Europe.
Methods: Of 18 countries, antibiotic susceptibility testing (AST) data for aminopenicillins, 3rd generation cephalosporins, aminoglycosides and fluoroquinolones of primary invasive *E. coli* isolates were extracted from the EARSS database for 2002. ESAC provided total antibiotic consumption and consumption of the antibiotic classes penicillins, cephalosporins, aminoglycosides, and fluoroquinolones in ambulatory care according to ATC/DDD classification for 2001. For statistical analysis a Spearman rank correlation (Rs) test was used.
Results: A significant correlation ($P < 0.01$) was found between resistance to aminopenicillins (Rs = 0.740), aminoglycosides (Rs = 0.680), fluoroquinolones (Rs = 0.697) and consumption for the same antibiotic classes, except for consumption and resistance to 3rd generation cephalosporins (Rs = 0.401, $P > 0.05$). Probably because 3rd generation cephalosporin resistance is mainly a hospital problem (outbreaks of ESBL positive strains) it is more related to infection control and not reflected by consumption in ambulatory care. Aminopenicillin (Rs = 0.740) and aminoglycoside resistance (Rs = 0.562) both correlated ($P < 0.05$) with consumption of broad-spectrum penicillins, and may indicate genetic linkage. Aminopenicillin (Rs = 0.585) and fluoroquinolone resistance (Rs = 0.534) both correlated with total ambulatory care consumption ($P < 0.05$).
Conclusions: At this high aggregation level a lot of unknown and uncontrollable factors can lead to confounding. However, correlations were found between antibiotic resistance in *E. coli* and antibiotic consumption in ambulatory care, especially by antibiotic class, signalling the presence of effects worthy of further ecological investigations.

P1199 Faecal carriage of quinolone-resistant *E. coli* in children in the community

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Objectives: To define the prevalence and risk factors for faecal carriage of quinolone resistant *E. coli* strains in children in the community and to evaluate the mechanisms of resistance.
Methods: A cross-sectional study of children visiting their paediatricians. *E. coli* was isolated from the stool of each child, obtained by rectal swabs. Epidemiological data was gathered from the patient's file and parent questionnaire. Risk factors for carriage of quinolone-resistant strains were assessed by univariate and multivariate analyses. Resistance to nalidixic acid (NA) and ofloxacin (OFL) was established by the disc diffusion assay, and confirmed by MIC determination, using the agar dilution method. Activity of efflux pumps in the NA-R strains was assessed with reserpine, an efflux pump inhibitor. DNA sequencing identified mutations in strains lacking efflux pump activity.
Results: 812 rectal swabs were obtained from children aged 0–40 months. A total of 627 of the cultures (77%) yielded *E. coli* strains. 133/627 (21%) of the isolates were NA-R. 32/133 (24%) of the NA-R strains were also OFL-resistant (OFL-R). The NA-R isolates were significantly more resistant to all other antimicrobials. The only risk factor for carriage of NA-R strains was younger age (adjusted OR 3.12; 95%CI 1.85–5.28). Efflux pump activity was found in 75 NA-R strains (44%), yet only in three OFL-R strains (7%). Point mutation analysis of 20 NA-R strains showed that most of the NA-R/OFL-R strains ($n = 8$) exhibited three specific mutations: gyrA83 – 100%, gyrA87 – 100%, and parC – 75%. However, NA-R/OFL-S isolates ($n = 9$) displayed fewer mutations: gyrA83 – 89%, gyrA87 – 78%, and parC – 0%.
Conclusions: Quinolone-resistant *E. coli* is prevalent in children despite no previous therapy with quinolones. Apart from young age, no risk factors were found for faecal carriage of NA-R strains by children. Efflux pump activity was associated with resistance to NA, but not to OFL. Multiple target mutations were correlated with higher-level resistance to quinolones.

P1200 Laboratory and hospital reference data for validation of resistance data collected by the European Antimicrobial Resistance Surveillance System (EARSS)

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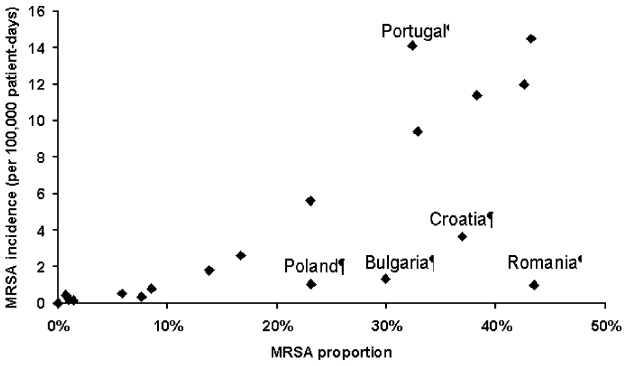
Objectives: The European Antimicrobial Resistance Surveillance System (EARSS) collects antimicrobial resistance data for five invasive indicator pathogen species (*Streptococcus pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecium* and *E. faecalis*). Data are collected from almost 800 laboratories in 28 countries from 1999 onwards. A questionnaire was mailed to all laboratories to assess the representativeness and validity of data collected by EARSS and to calculate specific incidence rates.

Methods: In the spring of 2003, a questionnaire with laboratory- and hospital-specific questions was mailed via national distributors to participating laboratories. Information was collected on geographic location, blood culture frequency, hospital type, and degree of specialisation, catchment population, number of beds and number of patient-days. With this information, we calculated population coverage and the incidence of the infections monitored by EARSS.
Results: The questionnaire was sent out in 22 of the 28 countries participating in EARSS. In these countries, 306 of 379 laboratories responded (81%). EARSS covered on average 70% of the national population of these countries, which amounts to almost 94 million inhabitants. In most countries, laboratories were evenly distributed over the country. The number of blood culture sets per 1000 patient-days varied over Europe, being less than 15 in the Eastern European countries and over 30 in most Northern European countries, as well as in the United Kingdom, Spain, France and Israel. Plotting country-specific MRSA proportions against incidences showed a high linear correlation (R^2, 0.92) for almost all countries, except for some Eastern European countries (Figure). This is possibly due to the culturing of serious infections only, leading to an overestimation of MRSA proportion, but an underestimation of MRSA incidence.



Conclusion: Good national coverage indicates that the EARSS data give a good approximation of the situation of antimicrobial resistance in Europe. EARSS susceptibility proportions correctly reflect resistance incidence in most countries, although resistance might be overestimated in some Eastern European countries.

Vaccines and immunisation

P1201 Modulation of immune response on ovalbumin sensitisation in BCG immunised mice

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Introduction: Epidemiological studies suggest an inverse correlation between infections and development of atopy. The aim of this study was to test whether a pre-existing Th1-type immune response elicited by BCG immunisation could suppress allergic sensitisation in Balb/c mice and whether such immunomodulation is depended on immunogenicity of used BCG substrains.
Methods: Immunisation: Male Balb/c mice, aged 6–8 weeks were immunised with an attenuated *Mycobacterium bovis* strains (BCG): Moreau or Tice commonly used as BCG vaccines in infants. 1×10^6 CFU/0.1 mL PBS were injected in each mouse intravenously. Control mice received PBS. Sensitisation: Half of immunised mice were sensitised by repeated intraperitoneal injections of 10 μg chicken ovalbumin (OVA) emulsified in 1.5 mg $Al(OH)_3$ on days 14, 28 and 35. Control mice were injected with $Al(OH)_3$ alone. Tissue culture of spleen mononuclear cells: Spleens from all animals were prepared on day 42. Cells (MNCs) were stimulated for 96 h with Concanavalin A (2.5 μg/mL) or 10^4 BCG/mL or 20 μg/mL OVA. Measure of lymphocytes proliferation: To some of the cultures 3[H]-thymidine was added for the last 18 h (1 μCi/well) and the proliferation rate was calculated. Determination of cytokines level: IL-4 and IFN-γ were measured by ELISA (OptEIA-kits) in supernatants of MNCs cultures.
Results: (1) Splenic MNCs of BCG (Moreau or Tice) immunised mice after activation with Con A have shown higher proliferation rate (SIM = 14.3; SIT = 19.7) than, splenic MNCs of BCG immunised and after then sensitised OVA mice (SIM = 1.61; SIT = 2.11) or only OVA sensitised mice (SIM = 5.55). The differences were significant ($P < 0.001$). (2) Significantly higher level of IFN-γ production was observed in MNCs cultures of mice immunised BCG (Moreau = 3.4 ng/mL; Tice = 4.4 ng/mL) than in MNCs cultures of mice OVA sensitised (2.5 ng/mL). (3) The animals that were immunised BCG and then sensitised OVA have shown increase of production IFN-γ (Moreau = 3.2 ng/mL; Tice = 3.8 ng/mL). (4) Production of IL-4 was significantly higher in MNCs cultures of animals sensitised OVA (0.4 ng/mL) than of animals immunised BCG (Moreau = 0.2 ng/mL; Tice = 0.27 ng/mL) or immunised BCG and then sensitised OVA (0.25 and 0.29 ng/mL).
Conclusions: BCG vaccine can modulate immune response of mice on OVA changing it from Th2-type to Th1. Immunogenicity of used BCG substrain has influence on intensity of Th2 immune response inhibition.

P1202 Polyprenyls of plant origin augment specific biologic activity of tick-borne encephalitis and rabies vaccines

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Objectives: This study was aimed to reveal possible potentiating effect of Phosprenyl compound (PC) upon specific biologic activity of tick-born encephalitis (TBEV) and rabies (RV) vaccines. The main active ingredients of PC are polyprenyls extracted from pine needles.
Methods: Vaccines effectiveness was estimated using standard protective murine assay developed at the IPVE. Each group consisted of 15 BALB/c mice, 12–14 g. Concentrated tick-born encephalitis vaccine (TBEV) series 494 and 552, and rabies vaccine (RV) series 429, 430 were used throughout. PC was used as a 0.4% solution. In the experiments with RV, the PC drug was injected i.m. at a dose 25 mcg/mouse (10 RV dilutions from 1:5 to 1:10 000 were used). In the experiments with TBEV, PC was diluted so that any dilution of the vaccine would contain 100 mcg PC, and this admixture was injected s.c. Control mice were immunised with the vaccines only. RV or TBEV was injected twice with 1-week interval. Seven days later mice were infected with either of the two viruses. TBE virus was injected i.p., at a dose 100 LD50/0.2 mL, and rabies virus s.c., at a dose 600 LD50/0.1 mL). Simultaneously with each of the vaccine, mice were injected with PC according to two protocols: when injected with TBEV, to each of the vaccines dilutions 100 mcg of PC was added and injected in one syringe; when vaccinated with RV, mice were also injected with PC (i.m. 25 mcg/mouse). After infection, all mice were supervised for 3–4 weeks and numbers of ill and dead mice were estimated. Index of mortality, and 50% minimal immunising dose (MID50) were then calculated.
Results: PC was found to significantly increase specific protective activity of RV: when injected simultaneously with PC, index of

RV's protective activity was increased 1.9-fold compared with immunisation protocol without PC. Similarly, MID50 of TBEV injected simultaneously with PC was augmented 8–10-fold in comparison with the control.
Conclusion: Taken together, our data prove that PC is able to potentiate specific biologic activity of viral vaccines against tick-born encephalitis and rabies. The effect of PC was observed following both i.m. and s.c. injections. Possible mode of the above PC action may be connected with the earlier revealed ability of PC to activate mononuclear phagocyte cells at early stages of the immune response development, and to augment IL-1, IL-4, IL-5, and IL-6 production. Adjuvant effect of PC for RV in dogs was also revealed in our previous studies.

P1203 The assessment of 3 years of routine vaccination against *Haemophilus influenzae* b in the Czech Republic

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Objectives: Routine vaccination against *Haemophilus influenzae* b (Hib) of infants using tetravalent DiTePe+Hib vaccine was introduced in the Czech Republic in July 2001. Three years before the introduction of this mass vaccination the nation-wide active surveillance of invasive diseases caused by Hib was introduced. These conditions provided an excellent possibility to assess the impact of routine vaccination against Hib.
Methods: The active surveillance was introduced in January 1999 and case definition of invasive Hib disease included meningitis, epiglottitis, bacteraemia and/or sepsis, pneumonia and arthritis. According to the laboratory results, Hib cases were defined as confirmed, probable and suspected. After the introduction of routine Hib vaccination of infants, the surveillance was extended by the reporting of Hib vaccination failure (three categories according to the classification used in the UK): true vaccine failure, apparent vaccine failure and possible vaccine failure.
Results: A total incidence of Hib invasive disease ranged between 1.0 and 1.1/100 000 population before the introduction of routine Hib vaccination (in 1999 and 2000). The highest incidences in both years were in the age groups 0–11 months (17.1/100 000, and 15.6/100 000, respectively) and 1–4 years (17.4/100 000, and 20.9/100 000, respectively). The active surveillance data for 2001–2003 indicate the decrease of Hib invasive disease in the target age group (0–11 months) after the introduction of routine Hib vaccination, in which the age specific morbidity was 15.6/100 000 in 2001, 3.3/100 000 in 2002 and 3.3/100 000 in 2003 (preliminary data for 2003). Data of vaccine failure are as follows: one case of possible vaccine failure in 2001, no vaccine failure in 2002 and two true vaccine failures in 2003 (preliminary data for 2003).
Conclusion: Routine Hib vaccination of infants was introduced in the Czech Republic in July 2001. The results of active surveillance indicate rapid decrease of Hib invasive disease in the target age group under the influence of this mass vaccination. Hib vaccine failure is very rare.
Acknowledgement: This study was supported by research grant NI/6803-3 of the Internal Grant Agency of the Ministry of Health of the Czech Republic.

P1204 Changes in distribution of pneumococcal serotypes in the Czech Republic between January 1996 and December 2003

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Objectives: Any project to develop and/or introduce an appropriate narrow spectrum conjugate pneumococcal vaccine implies collecting serotype data from different countries all over the world. We have reviewed the capsular serotypes/serogroups of pneumococci identified from invasive diseases in the Czech Republic (CR) between January 1996 and December 2003.
Methods: Pneumococci isolated from patients with invasive diseases were typed using the quellung reaction with antisera from the Statens Serum Institut, Copenhagen.
Results: A total of 1531 strains from blood, cerebrospinal fluid (CSF), section material (1022 strains), pleural aspirate, bronchial aspirate and bronchoalveolar lavage fluid (509 strains) were examined; 239 out of them were collected from young children (under 5 years), and 1292 from older children and adults (5 years or more). In descending order of frequency, types 3, 19F, 23F, 1, 14 and 4 were common to all age groups, while types 6B (13%), 19F (12%), 14 (11%), 1 and 23F (8% each), 3 (7%) and 6A (5%) were significantly associated with young children and types 3 (14%), 19F (8%), 1, 14 and 23F (6% each) and 4 and 8 (5% each) were prevalent in older children/adults. The coverage of the 7, 9 and 11-valent conjugate vaccines was substantially higher in children as young as 5 years with 63, 70, and 80%, respectively, compared with 35, 40, and 59% in the other age group. We investigated changes in pneumococcal serotypes distribution between the years 1996 and 2003. The proportion of pneumococcal infections caused by the seven serotypes included in the conjugate vaccine increased significantly from 56 to 72% in young children, and from 22 to 39% in older children/adults. The proportion of infections caused by the epidemic serogroups (1–3 and 5) in patients regardless of age decreased slightly from 20 to 16%. During the period studied, a progressive increase in the incidence of strains of serogroup 4 from 2 to 9% was recorded. Strains of serogroup 5 were detected rarely, serogroup 2 was not identified.
Conclusion: Although a significant increase in the incidence of serotypes/serogroups included in the so far only licensed 7-valent conjugate pneumococcal vaccine was found in the CR for the period 1996–2003, the 11-valent conjugate formulation only remains suitable for use among the Czech population. Serogroups 1 and 3 regularly prevail in clinical specimens of all types collected from patients with invasive infections of all age groups.

P1205 Efficacy of a vaccine against *Salmonella enteritidis* based on Gantrez nanoparticles as adjuvant and delivery system

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Objectives: The most commonly used adjuvants for human use are aluminium salt derivatives. However, they present some drawbacks including irritation and inflammation, undesirable IgE production (strong Th2 response) and incapacity to elicit effective Th1 cellular immune response. Other adjuvants, including Freund's adjuvant, primarily induce a Th1 response but the side effects are not acceptable for human use. In the current communication, we introduce nanoparticles of Gantrez as an alternative adjuvant.
Methods and results: *Salmonella enterica* serovar Enteritidis (*S. Enteritidis*) is a major cause of human food-borne illness. The development of stable, efficacious, and safe live vaccines to prevent human and poultry salmonellosis is still a major challenge. Live attenuated vaccines administrated parenterally are protective and used widely in poultry. However, they cause some inconvenience related with their residual virulence and instability related with storage. An alternative is the employment of subcellular vaccines containing immunodominant components of the bacteria and the appropriate safe immunoadjuvant. Accordingly, an antigenic extracts from *S. enteritidis* (clinical isolated) was obtained after a heat treatment in saline of whole bacteria [HE extract, containing flagellin, porins, OmpA, fimbriae (14–22 kDa), among other proteins, and LPS]. In order to improve the immunogenicity, HE was loaded in Gantrez nanoparticles of a size close to 200 nm. Chemical and serological analysis indicated that HE encapsulated conserved its antigenicity. The protection conferred by immunisation with free HE or HE-Gantrez nanoparticles against a lethal dose of *S. enteritidis* in BALB/c mice was similar (90 vs. 80%, after 21 days post-challenge). By contrast, empty nanoparticles conferred only 20% of protection, and all control mice died after 6 days post-challenge. Spleen cells from immunised mice with HE-nanoparticles elicited a higher level of IFN-gamma with

respect to free HE (801 vs. 373 pg/mL, respectively, on day 10 post-immunisation); furthermore, the specific humoral immune response induced corresponded with an enhanced Th1 response as well (IgG2a/IgG1, 4.51 vs. 2.98, respectively).
Conclusions: Nanoparticles of Gantrez provide a safe and easily manufactured vaccine adjuvant and delivery system. Further studies in new experimental conditions are now in progress in order to confirm the beneficial properties of this new adjuvant.

P1206 The effects of gene deletions in recombinant vaccinia virus (rVV) genome on anticancer immunity induced by rVV based vaccine

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Objectives: The association between human cervical carcinoma and infection with high-risk human papillomaviruses (e.g. HPV16, HPV18) has been proved formerly. A therapeutical vaccine effectively enhancing cell-mediated immune responses against tumour-associated antigens would offer a great potential for the treatment of cervical cancer. Our aim was to examine the role of B8R, A44L, C7L and C23L/B29R rVV genes for immunogenicity of the model vaccine based on recombinant vaccinia virus expressing modified HPV16 E7 tumour antigen.
Methods: Recombinant viruses were derived from strain Praha, clone 13. Deletion mutants were prepared by homologous recombination with DNA of specially constructed plasmids. HPV16 E7 gene carried three point mutations in Rb-protein binding domain (rVV-E7ggg). Dendritic cells were derived from C57Bl/6 mice bone marrow and cultivated in presence of GM-CSF cytokine. The immune response was detected by IFN-gamma ELISPOT and tetramer assays. Anticancer immunity was shown as tumour growth protection after challenge with tumorigenic TC-1 cells.
Results: (1) Dendritic cells supported protective effects of rVV-E7ggg immunisation. (2) *In vitro* tests of cell-mediated immunity showed that after i.p. administration, there was no difference between B8R (encodes soluble IFN-gamma receptor) deleted virus and virus without deletion. (3) B8R gene deletion increased immunogenicity of intraperitoneally administered rVV in *in vivo* experiment. (4) Viruses with A44L or C23L/B29R (soluble receptor for CC chemokines) deleted genes induced lower cellular response. (5) A44L (3-beta hydroxysteroid dehydrogenase) gene deletion did not influence the protection after rVV i.p. immunisation. (6) B8R, A44L and C7L (host-range protein) gene deletions did not affect immunogenicity of rVV transduced dendritic cells.
Conclusions: Our results showed that products of B8R, A44L or C7L genes do not affect immunogenicity of dendritic cell-based vaccines.

Antimicrobial susceptibility in Gram-negative bacteria - II

P1207 Simple method to evaluate the *in vitro* activity of several antimicrobial agents against bacterial biofilms

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Objectives: The use of prosthesic materials for temporary or permanent implantation has been accompanied by the emergence of implant-associated infections, which are difficult to eradicate. Standard *in vitro* susceptibility tests only evaluate antimicrobial agent activity against planktonic bacteria and do not evaluate adherent bacteria. In the present study we have developed a simple and reproducible method to evaluate *in vitro* bactericidal activity of several antimicrobial agents against sessile bacteria. Moreover, bactericidal activities of these antimicrobial agents against planktonic and adherent bacteria have been evaluated.
Methods: *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) were used. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were determined by broth microdilution method, according to NCCLS guidelines. The antimicrobial agents tested were: ampicillin (AMP), cefuroxime (CFX), cefotaxime (CTX), ciprofloxacin (CIP), gentamicin (GNT), trimethoprim/sulfamethoxazole (SXT), imipenem (IMP) and meropenem (MRP) against *E. coli*, and piperacillin (PIP), CIP, GNT, tobramycin (TBM), ceftazidime (CTZ), cefepime (FEP), IMP and MRP, against *P. aeruginosa*. MBC of adherent bacteria (MBCADH) was determined from the 96-well plates used for MIC and MBC determinations. Plates were washed two times with cold PBS. At this time, 150 μL of PBS were added to each well, the plates were sealed and adherent bacteria were detached by sonication. Surviving bacteria were determined by plating. All experiments were performed five times.
Results: With *E. coli* the MBCADH values of AMP, CFX and CTX were 32, 512 and 64 times higher than MBC of suspended bacteria, respectively. Carbapenems-MBCADH values were 512 times higher than MBC. MBCADH values of CIP, GNT and SXT were 32, 64 and 256 times higher than MBC, respectively. With *P. aeruginosa*, MBCADH values of PIP, CTZ, FEP and both carbapenems were 256 times higher than MBC. CIP-MBCADH values were also 256 times higher than MBC and aminoglycosides-MBCADH values were 512 times higher than MBC.
Conclusion: The developed method is simple and reproducible to evaluate *in vitro* activities of tested antimicrobial agents against sessile bacteria. For all antimicrobial agents tested, and with both strains, the MBC of adherent bacteria was always much higher than MBC of suspended bacteria.

P1208 Antibiotic resistance markers (ARMS) in the *Bacteroides fragilis* group (Bfg): Clindamycin (CL), Cefoxitin (FOX), and Trovafloxacin (TV) alone and in combination

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Objectives: Antimicrobial resistance in Bfg has continued to increase to several classes of agents, but little is known about multiresistance among Bfg. This study determined the effect of selected ARMS as phenotypic predictors of cross-resistance (C–R) to other antimicrobials.
Methods: A total of 3100 clinical isolates of the Bfg were tested for susceptibility to CL, FOX, TV, cefotaxime (TX), ceftizoxime (ZX), cefotetan (TN), piperacillin (PP), ampicillin-sulbactam (AS), piperacillin-tazobactam (PT), imipenem (IM), meropenem (ME) and ertapenem (ER). MICs were determined by NCCLS broth microdilution using the same test medium throughout. MICs were collated to determine percentage of isolates susceptible (S) or resistant (R). Statistical comparisons (odds ratios (OR) and adjusted *P*-values) were established by Pearson's chi-squared test followed by Bonferroni stepdown adjustment.
Results: Overall % Cl and % Fox-R were 22 and 16%, respectively, regardless of C–R to other agents, however, Cl-R was 13% in the absence of Fox-R and Fox-R was 4% in the absence of Cl-R ($P < 0.05$). When CL and FOX MICs were phenotypically linked (SS to RR) increased C–R (%R) to other agents was noted for ZX (9–52%; $P < 0.01$; OR, 2–11); TX (12–76%; $P < 0.01$; OR, 2–23); TN (10–93%; $P < 0.01$; OR, 4–125); PP (6–68%; $P < 0.01$; OR, 3–35); AS

(0.3–22%; $P < 0.001$; OR, 8–103); and TC (0.4–26%; $P < 0.01$; OR, 5–91). Neither CL nor FOX resistance increased the % R to PT while both IM and ER but not ME were influenced by FOX and CL ($P < 0.05$) alone and together. TV-R was 6% overall and 4% without CL-R and FOX-R isolates. C-R was more significant when TV-R was linked to FOX-R. Only two isolates had a CL FOX TV-R phenotype and were C-R to ZX, TX, TN, PP, AS, TC and ER but S to PT.
Conclusions: Results demonstrate significant associations among ARMS of three separate classes, which predict C-R to beta-lactams among Bfg. These analyses of phenotypic antibiotic resistance suggest further investigation of genetic linkage and/or clinical outcomes.

P1209 *In vitro* activities of BAL9141 and seven other beta-lactam antimicrobial agents towards clinical isolates of 12 members of the Enterobacteriaceae family

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Objectives: BAL9141 is the first of a new class of anti-MRSA cephalosporins, which also possesses broad activities towards most clinically relevant bacterial pathogens. This study aimed at evaluating the *in vitro* activity of BAL9141 and seven other beta-lactams against selected Enterobacteriaceae isolates including extended spectrum beta-lactamase (ESBL)-producing strains.
Methods: A total of 209 isolates comprising 15 *Citrobacter freundii*, 20 *Enterobacter aerogenes*, 30 *Enterobacter cloacae*, 26 *Escherichia coli* (11 of which were known to be ESBL-producers), 16 *Klebsiella oxytoca* (five ESBL-producers), 42 *Klebsiella pneumoniae* (28 ESBL-producers), and 10 each of *Citrobacter koseri*, *Morganella morganii*, *Providencia rettgeri*, *Providencia stuartii*, *Serratia liquefaciens*, and *Serratia marcescens* were obtained from various culture collections in Germany. MICs of BAL9141, cefepime, ceftazidime, ceftriaxone, aztreonam, piperacillin, piperacillin-tazobactam, and imipenem were determined by broth microdilution according to NCCLS guidelines.
Results: BAL9141 displayed excellent activity against the majority of isolates, but low activities towards ESBL-producing *E. coli* and *Klebsiellae*. Activity of BAL9141 against individual species based on MIC50- and MIC90 values (mg/L) and rates of susceptible strains at the proposed breakpoint of 4 mg/L were as follows: *C. koseri* (0.06/2/90%), *C. freundii* (0.06/0.5/100%), *E. aerogenes* (0.06/0.125/95%), *E. cloacae* (0.125/2/93%), *E. coli* [overall: 0.125/64/65%; known ESBLs: 64/128/18% others: 0.06/0.125/100%], *K. oxytoca* (2/>64/56%), *K. pneumoniae* [overall: 4/64/50%; known ESBLs: 32/64/25%; others: 0.125/0.5/100%], *M. morganii* (0.06/0.06/100%), *P. rettgeri* (<0.06/<0.06/100%), *P. stuartii* (<0.06/<0.06/100%), *S. liquefaciens* (0.125/1/100%), and *S. marcescens* (0.125/0.25/100%). Overall, 80.4% of isolates were inhibited by BAL9141 at 4 mg/L. This rate was comparable to those of ceftriaxone (79.4%), ceftazidime (77.5%), and aztreonam (78.9%).
Conclusion: In addition to its anti-MRSA activity, the spectrum of activity of BAL9141 towards members of the family Enterobacteriaceae resembles that of third generation cephalosporins and aztreonam.

P1210 Drug resistance patterns of typhoidal and non-typhoidal *Salmonella* species in Hamadan, west of Iran

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Objectives: The epidemiological studies indicate that the incidence of salmonellosis is increasing throughout the world. The uncontrolled and inappropriate usage of antibiotics has caused multidrug resistance in these organisms, in recent years.
Methods: In a cross-sectional descriptive study, 204 strains of typhoidal salmonella (T.S) and 114 strains of non-typhoidal salmonella (N.T.S) were examined to determine drug resistance. The strains were collected from patients who referred to cilinical centres in Hamadan during 1999–2001. They were serotyped and then tested for their antibiotic resistance patterns, using Kirby–Bauer method for eight antibiotics.
Results: The salmonella isolated from patients were as follows: '*S. typhi*, *S. paratyphi* A, B, C. *S. typhimurium*, *S. enteritidis*, *S. cholerasuis*, *S. agona*, *S. arizona*, *S. infantis*, *S. havana*, *S. lexington* and *S. virchow*'. A proportion of strains (>60%) were resistance to carbenicillin and ampicillin. Resistance to ciprofloxacin and nalidixic acid was very low (<15%). *S. typhimurim* (100%), *S. typhi* (95.7%) paratyphi B (89.2%) and enteritidis (60%) showed multidrug resistance (MDR).
Conclusions: Our results showed that most of *Salmonella* spp. isolated from patients in Hamadan city (the west of Iran) was resistant to beta-lactam antibiotics, whereas, most of them were sensitive to fluoroquinolones antibiotics. We suggest that the use of some newer antibiotics such as new fluoroquinolones, ceftazidime and aztreonam as effective therapy against salmonella species in this region.

P1211 Efficacy of crude extracts of Thai medicinal plants on antibiotic-resistant *Helicobacter pylori* strains isolated from peptic ulcers

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Objectives: *Helicobacter pylori* resistant rates to both metronidazole and clarithromycin are now increasingly reported. The aim of our study was to screen for effective medicinal plants widely used in Thai traditional medicine for the treatment of this organism.
Methods: Twenty-four preparations of aqueous and ethanolic extracts of 12 kinds of Thai herbs including *Andrographis paniculata*, *Centella asiatica*, *Curcuma longa*, *Garcinia mangostana*, *Peltophorum pterocarpum*, *Piper betle*, *Psidium guajava*, *Punica granatum*, *Quercus infectoria*, *Uncaria gambir*, *Walsura robusta*, and *Zingiber cassumunar* were tested for their antibacterial activity against 17 hospital strains of antibiotic-resistant *H. pylori*, *H. pylori* ATCC 43504, and *H. pylori* ATCC 43579. Inhibition of growth was preliminarily tested by the paper disc agar diffusion method. Antibiotic susceptibility discs were used as control. Minimum inhibitory concentration (MIC) was determined by the agar dilution method in petri dishes with millipore filter.
Results: All clinical isolates tested proved susceptible to six medicinal plants including *P. pterocarpum*, *P. betle*, *P. granatum*, *Q. infectoria*, *U. gambir*, and *W. robusta*. The inhibition zones (annular radius) ranged from 9 to 23 mm. Among these extracts, *P. granatum*, *Q. infectoria*, and *W. robusta* demonstrated the greatest inhibition zones, ranging from 15 to 23 mm. Both aqueous and ethanolic extracts of *P. granatum* and *Q. infectoria* posessed significantly effective antibacterial activity against all strains of *H. pylori* with the MIC values of 0.8 and 6.25 mg/mL, respectively.
Conclusions: As both aqueous and ethanolic extracts of *P. granatum* and *Q. infectoria* were very effective against all strains of *H. pylori*, these two plant species are being investigated in this laboratory to provide alternative treatment of *H. pylori*.

P1212 French multicentre study of antibiotic resistance among Gram-negative anaerobes. Focus on decreased susceptibility to metronidazole

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Objectives: Survey of antibiotic resistance among Gram-negative anaerobes.
Methods: MICs for amoxicillin, ticarcillin either alone or combined with clavulanic acid, piperacillin–tazobactam, cefoxitin, imi-

penem, clindamycin and metronidazole were determined using the agar reference method (NCCLS, Norma M11A5) on 376 strains collected in nine French hospitals in 2002–2003.
Results: Within the *Bacteroides fragilis* group, resistance rates (NCCLS breakpoints) were, respectively: amoxicillin + clavulanic acid 4.3%, ticarcillin 29.2%, ticarcillin + clavulanic acid and piperacillin + tazobactam 0.2%, cefoxitin 6.3%, imipenem 0.08%, clindamycin 38.5%. Resistance to metronidazole could not be detected but 3.5% of the investigated strains demonstrated decreased susceptibility to metronidazole (MIC = 8 or 16 mg/L). The same phenomena could be observed for 7/65 strains of *Prevotella*. β-lactamase production was detected in 55% of *Prevotella* strains and 5.4% of *Fusobacterium* strains. Considering Gram-negative bacilli other than the *B. fragilis* group resistance to piperacillin-tazobactam, cefoxitin and imipenem could not be detected. On the whole Gram-negative anaerobes resistance to clindamycin reached nearly 30%, meanwhile 4.5% of the investigated strains had decreased susceptibility to metronidazole.
Conclusion: Resistance rates were similar to those obtained in 2000–2001 but decreased susceptibility to metronidazole appeared for the first time among *Prevotella* species.

P1213 Rate of capnophilic Gram-negative bacteria isolated from patients with periodontitis and study of their sensitivity to selected antibiotics

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Objectives: Microbiological studies identified more than 200 bacterial species in periodontal pockets, only a limited number have been implicated as periodontal pathogens. The purpose of this study was to investigate the rate of capnophilic Gram-negative bacteria in patients with periodontitis and to determine the sensitivity of isolates to selected antibiotics.
Methods: Samples were collected with sterile paper points from deepest periodontal pockets of 406 patients (161 males, 245 females; aged 18–55 years). The samples were cultured under capnophilic conditions on selective media. Isolates were characterised to species level by conventional biochemical tests. The sensitivity of isolates to antibiotics was investigated by Kirby–Beuer method.
Results: The rate of isolates was 186 *Actinobacillus actinomycetemcomitans* (45.8%), 152 *Capnocytophaga* species (37.4%) and 139 *Eikenella corrodens* (34.2%). The rate of samples associated with monobacteria and polybacteria were 295 (72.7%) and 85 (20.9%), respectively. Capnophilic Gram negative bacterial growth was not observed in 32 samples (7.9%). The sensitivity of these bacteria to ampicillin, was 67.1, 73.3, 44.2%; Chloramphenicol 94.9, 83.4, 90.7%; doxycyclin 90.9, 87.5, 86.4%; erythromycin 50.9, 86.8, 49.7%; tetracycline 94.9, 84.7, 78.6% and penicillin G 64.8, 70.5, 54.1%, respectively. The results of statistical analysis (Chi-square test) show no significant differences between sex ($P > 0.60$) and also age groups of patients ($P > 0.70$).
Conclusion: It is concluded that, in the number of patients, samples, and the complexity of capnophilic Gram-negative bacteria and in other samples only one of these bacteria were identified. Further studies for identification of other agents are suggested. Meanwhile, the sensitivity of isolates to selected antibiotics was relatively high.

P1214 *In vitro* activity of colistin against non-fermentative Gram-negative bacilli

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Objectives: Multi-drug resistance of Gram-negative nosocomial isolates has become a problem in everyday clinical practice in the intensive care units. Because no fundamentally new anti-infective drugs are currently available, it appears that we need to re-evaluate the 'old' drugs. Colistin, a polymyxin was used from the 1960s to the early 1980s. Because of toxicity considerations its systemic use is completely abandoned. The aim of this study was to evaluate the *in vitro* activity of colistin against non-fermentative Gram-negative bacilli of which are difficult-to-treat nosocomial pathogens.
Materials and methods: A total of 132 non-fermentative Gram-negative isolates (55 *Pseudomonas aeruginosa*, 48 *Acinetobacter baumanii*, 21 *Stenotrophomonas maltophilia*, eight *Burkholderia cepacia*) were tested. Colistin was obtained from Sigma Chemicals and Co. Minimal inhibitory concentrations (MICs) were determined using agar dilution technique.
Results: The antimicrobial activity of colistin against the Gram-negative nosocomial isolates are summarised in the Table.

Table. The minimum inhibitory concentrations (MICs) of colistin against the non fermentative gram-negative bacteria tested

Species (No. of isolates tested)	MIC (mg/L)		
	Range	MIC_{50}	MIC_{90}
A. aeruginosa (55)	2–≥32	2	4
A. baumanii (48)	≤0.5–≥32	2	4
S. maltophilia (21)	4–≥32	4	≥32
B. cepacia (8)	4–≥32	≥32	≥32

Conclusion: The results of this study are in broad agreement with other published data and confirmed that colistin has maintained useful *in vitro* activity against *P. aeruginosa* and *A. baumanii*. Colistin may be a good therapeutic option in the treatment of severe infections caused by multi-drug resistant *P. aeruginosa* and *A. baumanii*.

P1215 Susceptibility testing of *Stenotrophomonas maltophilia*: effect of temperature and medium on results

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Objective: Susceptibility testing of *Stenotrophomonas maltophilia* is particularly difficult and is affected by both temperature and medium. The purpose of this study was to identify suitable test methods and, if possible, interpretive criteria for appropriate antibiotics.
Methods: Seventy isolates of *S. maltophilia* were tested for susceptibility to co-trimoxazole, minocycline, moxifloxacin, co-amoxiclav and aztreonam. MICs were determined on IsoSensitest and Muller–Hinton agars at both 37 and 30°C. Disc diffusion zone diameters were measured on IsoSensitest agar at both temperatures. In addition chloramphenicol, ciprofloxacin and doxycycline were tested by disc diffusion only at 30°C.
Results: In general, isolates grew better at 30°C and MICs were higher and zone diameters smaller. MICs of co-trimoxazole were similar on both media at both temperatures and correlation with zone diameters was good and microbiological breakpoints easy to establish. For the tetracyclines correlation between MIC and zone diameter was good but there were no isolates with high-level resistance and, as MICs were close to the breakpoint recommended for other species, it was difficult to establish zone breakpoints. For all beta-lactams MIC results were higher on Muller–Hinton than on Isosensitest agar and there was poor correlation with zone sizes at both temperatures. Most isolates were clearly resistant to chloramphenicol and ciprofloxacin but moxifloxacin had some potentially useful activity.
Conclusion: Susceptibility testing of *S. maltophilia* should be done at 30°C, the optimum temperature for growth. Co-trimoxazole can be tested on either Muller–Hinton or IsoSensitest agar and microbiological breakpoints established with confidence. The tetracyclines and moxifloxacin can also be tested on either medium but breakpoints are tentative because of the lack of isolates with high-

level resistance and distribution of MICs around the MIC breakpoints. Interpretation of results for beta-lactam antibiotics is difficult; MICs are much higher on Muller–Hinton agar than on IsoSensitest agar and there is little or no correlation between MIC and zone size and for this reason they should not be tested by disc diffusion until the clinical relevance has been assessed.

P1216 Bactericidal activity of five antimicrobial agents against *Campylobacter jejuni* tested by time-kill studies

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Objectives: To evaluate the bactericidal activity of two macrolides (erythromycin (MIC 2 mg/L) and azithromycin (MIC 0.06 mg/L)), ciprofloxacin (MIC 0.25 mg/L), gentamicin (MIC 1 mg/L) and tetracycline (MIC 1 mg/L) against *Campylobacter jejuni* ATCC 33560.
Methods: Time-kill studies were performed in Campylobacter Enrichment Broth (Bolton formula) incubated in a microaerobic atmosphere at 42°C. Bacteria were inoculated in broth medium to a final concentration of approximately 5 × 105 CFU/mL. Antibiotics prepared in sterile water were added to final concentrations of 0.25, 0.5, 1, 2, 4, 8 or 16 × MIC. Samples (0.1 mL) were removed at periodic intervals at 0.5, 0, 2, 5 and 18 h of incubation. Samples were serially diluted 10-fold and subsequently spread on 5% blood agar plates. After 1 or 2 days of incubation in a microaerobic atmosphere, the number of colonies was counted.
Results: A viability decrease of >1 log was not observed within the initial 5 h of incubation regardless of concentration above MIC of erythromycin and azithromycin. However, this bacteriostatic phase was followed by a strong dose-independent bactericidal activity of both agents. In contrast, gentamicin and ciprofloxacin demonstrated rapid, dose-dependent bactericidal activities with viability reductions after 2 h incubation of >3 log at 16 × MIC for both agents. Tetracycline exhibited bacteriostatic activity.
Conclusion: This study suggests that the activities of erythromycin and azithromycin, two first-line drugs for the treatment of campylobacteriosis are similar. Gentamicin and ciprofloxacin exhibited rapid and significant bactericidal effects and support the position of the former as the drug of choice for systemic *Campylobacter* infections.

P1217 The effect of subinhibitory concentrations (sub-MICs) of amikacin and ciprofloxacin on adherence of P-fimbriated *Escherichia coli* strains to human epithelial cells

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Objectives: The aim of this investigation was to examine the influence of sub-MICs (1/2, 1/4 and 1/8 MIC) of amikacin (AN) and ciprofloxacin (CIP) on adherence ability to P-fimbriated *E. coli* strains to human epithelial cells.
Methods: Two *E. coli* strains (315 and 5579) isolated from urinary tract infections were used. The MICs of AN and CIP for each strain in Mueller–Hinton broth were determined by using microdilution method. Strains were grown in Tryptic Soy Broth to stimulate the expression of pili. Human periurethral epithelial cells were collected from fresh urine. Equal volumes of epithelial cells and antibiotics treated bacterial suspension were mixed in tubes. Following incubation, unattached bacteria were removed from the suspension by centrifugation. The final epithelial cell pellets were dried on glass slides in air and then were May–Grunwald stained. The attached bacteria on 40 separate cells were quantified by direct light microscopy and adherence was determined as the mean number of bacteria attached per cell.
Results: The mean number of bacteria attached to epithelial cell before their exposure to sub-MICs of antibiotics (control) was 47.4 for *E. coli* 315 and 45.9 for *E. coli* 5579. After exposure to 1/2 MIC of CIP the mean number of bacteria per epithelial cell was only 5.5 (*E. coli* 315) and 8.25 (*E. coli* 5579). After exposure to 1/4 MIC of CIP the mean number of bacteria was 25.15 (*E. coli* 315) and 26.25 (*E. coli* 5579); after exposure to 1/8 MIC it was 30.7 (*E. coli* 315) and 30.6 (*E. coli* 5579). AN affected the bacterial adhesive capacity less than did CIP. The mean number of bacteria attached to epithelial cell was 36.8 (*E. coli* 315) and 40.8 (*E. coli* 5579) at concentration 1/2 MIC of AN. After exposure to 1/4 MIC and 1/8 MIC the mean number of bacteria (*E. coli* 315) per epithelial cell was 40.6 and 42.4, respectively. After exposure to 1/4 MIC and 1/8 MIC the mean number of bacteria (*E. coli* 5579) attached to one epithelial cell was 45.6 and 45.7, respectively. These values corresponded to the values observed in control.
Conclusions: Sub-MICs of antibiotics decreased capacity of *E. coli* to adhere to epithelial cells. Decrease of the number of bacteria attached to one epithelial cell was proportional to concentrations of antibiotics. The adhesive capacity of P-fimbriated *E. coli* strains was significantly decreased after exposure to sub-MICs of CIP.

P1218 Activity of meropenem against Gram-negative isolates from a Polish paediatric intensive care unit – part of the MYSTIC (Meropenem Yearly Susceptibility Test Information Collection) Programme, 1997–2003

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Objectives: The aim of this analysis was to assess the *in vitro* activity of meropenem (MEM) and eight other antibiotics against Gram-negative isolates from a Paediatric Intensive Care Unit (PICU).
Methods: Between 1997 and 2003, 740 Gram-negative isolates were recovered from a variety of specimens obtained from children hospitalised in the PICU of the Children's Memorial Health Institute, Warsaw, Poland. Isolates were identified using conventional methods. Minimum inhibitory concentrations (MICs) of MEM, imipenem (IPM), piperacillin + tazobactam (TAZ), cefotaxime (CTX), ceftazidime (CAZ), cefepime (CPE), gentamicin (GM), tobramycin (TM) and ciprofloxacin (CIP) were determined using the NCCLS agar dilution method.
Results: The collection of Gram-negative isolates included *Escherichia coli* ($n = 107$), *Enterobacter cloacae* ($n = 153$), *Klebsiella oxytoca* ($n = 58$), *Klebsiella pneumoniae* ($n = 133$), *Serratia marcescens* ($n = 29$), *Acinetobacter baumanii* ($n = 59$), *Pseudomonas aeruginosa* ($n = 154$) and other species ($n = 47$). Carbapenems, MEM and IPM, were active against >90% of isolates, with the exception of *P. aeruginosa*. CIP showed similar high activity. However, our results are from PICU in which CIP is not used except for very serious infections. The MIC90 (mg/L) of MEM was nearly identical in 1997 and 2003. It was equal to 0.03–0.125 for Enterobacteriaceae, 1.0 for *A. baumanii* and 8.0 for *P. aeruginosa*. The MIC90 of IPM was equal to 0.25–0.5 for Enterobacteriaceae, 1.0 for *A. baumanii* and 16 for *P. aeruginosa*. The overall order of activity of betalactams was MEM > IPM > CPE > TAZ > CAZ > CTX. GM and TM were active against 62.4 and 58.9%, respectively.
Conclusions: MEM and IPM were the most active antibiotics (>90% susceptibility) against the tested isolates, with no observed reduction in activity over 7 years. After the carbapenems, CIP was the most active antibiotic, but CIP is very rarely used in children.

P1219 *Stenotrophomonas maltophilia* clinical isolates in a tertiary care hospital: 5-year study

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Objective: To determine the incidence and the susceptibility to antimicrobials of clinical isolates of *Stenotrophomonas maltophilia* in a tertiary care hospital over a 5-year period.
Methods: During the 5-year period, December 1998 to November 2003, all *S. maltophilia* strains from clinical specimens were studied. The isolates were identified by commercial available Pasco system

(ID panels). Over the entire study period, susceptibility testing was performed by the broth microdilution method using the Pasco system (MIC panels) and the Etest strips to different antimicrobial agents according to the recommendations of the NCCLS guidelines. *Pseudomonas aeruginosa* ATCC 27853 was used as quality control.
Results: A total of 146 *S. maltophilia* strains were isolated from clinical samples. The 46 isolates belonged to ICU patients (31.5%) and 100 were from patients of 22 different wards. The incidence of *S. maltophilia* was increased over the time of study. The number of isolates (n) per year was: $n = 3$ (1998–1999), $n = 32$ (1999–2000), $n = 24$ (2000–2001), $n = 36$ (2001–2002), $n = 51$ (2002–2003). The source of specimens was: sputum (52), bronchial secretions and lavage (37), pus (18), urine (13), blood (10), body fluids (7), tip of central vein catheters (7), and CSF (2). Regarding the antibiotic susceptibility, 100% of the strains were susceptible to moxifloxacin, 91% were susceptible to cotrimoxazole, 89% to levofloxacin, 76% to ceftazidime, 74% to ciprofloxacin, 72% to piperacillin/tazobactam and 67% to trovafloxacin. All strains were resistant to imipenem and 99% of the strains were resistant to meropenem. A variety of susceptibility phenotypes was observed and molecular studies (PCR-PFGE) suggested that there were no epidemic clusters in the hospital.
Conclusions: *S. maltophilia* has been an increasing nosocomial pathogen in our hospital. Moxifloxacin was the most active fluoroquinolone between levofloxacin, trovafloxacin, ofloxacin and ciprofloxacin followed by levofloxacin. Cotrimoxazole is still highly active agent among the other antimicrobials in our clinical isolates.

P1220 Antimicrobial resistance in *Campylobacter* strains isolated from humans in Crete, Greece

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Background: Infections with *Campylobacter* species, particularly *Campylobacter jejuni* and *Campylobacter coli*, are recognised as one of the most common causes of bacterial diarrhoea in humans worldwide.
Objective: To determine the antimicrobial resistance rates of thermophilic *Campylobacter* strains isolated from diarrhoeal patients in Crete, Greece.
Methods: A total of 621 *Campylobacter* isolates from stool specimens of patients suffering from acute diarrhoeal infections during January 1992 and November 2003 were included. Identification was done according to standard microbiological methods. Antimicrobial susceptibility testing was performed by the disk diffusion method using Muller–Hinton agar supplemented with 5% sheep blood; the plates were incubated at 37°C for 24 h in a microaerophilic atmosphere. The following antibiotics were tested: erythromycin, tetracycline, trimethoprim/sulfamethoxazole, gentamicin, chloramphenicol, and ciprofloxacin.
Results: Six hundred and twenty-one strains of *Campylobacter* were studied. Of them, 493 (79.4%) were *C. jejuni* and 128 (20.6%) *C. coli*. Most isolates (71.8%) were resistant to trimethoprim/sulfamethoxazole. Resistance rates observed to other antibiotics were as follows: 43.6% to tetracycline, 7.4% to chloramphenicol, and 2.5% to gentamicin. High percentages of resistance to ciprofloxacin (39.6%) were found, while resistance to erythromycin was observed in 16.7% of the isolates.
Conclusion: The increased rates of *Campylobacter* resistance in our region emphasise the need for a more restrictive policy on the use of antibiotics in both humans and farm animals.

P1221 *In vitro* activities of non-traditional antimicrobials against multiresistant strains of *P. aeruginosa* and *A. baumanii* isolated from intensive care units

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Objective: To evaluate *in vitro* activities of azithromycin, doxycycline, rifampin and colistin against multi drug resistant (MDR) strains of *P. aeruginosa* and *A. baumanii* isolated from intensive care units (ICUs).
Materials and methods: Thirty-five *P. aeruginosa* and 25 *A. baumanii* stains that were found to be multi-drug resistant were included the study. Isolates were collected from the specimens of the patients in ICUs between 2001 and 2003. All isolates were identified by standard methods and stored at −20°C until use. Antibiotic powders of azithromycin (Pfizer), doxycycline (Sigma), rifampin (Sigma) and colistin (Sigma) were obtained from the manufacturers. MICs were determined by agar dilution method on Muller–Hinton agar and results were interpreted according to the recommendations of NCCLS for azithromycin, doxycycline and rifampin. MIC value for colistin was accepted <4 mg/mL (Catchpole et al, J Antimicrob Chemother 1997; 39: 255–260). *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922 were used as control strains. MIC50 and MIC90 values are summarised in the Table.

Table MIC50 and MIC90 values of tested antibiotics for multidrug resistant *P. aeruginosa* and *A. baumanii* strains

Bacteria (n)	Antibiotics	MIC50	MIC90	Range
P. aeruginosa (35)	Azithromycin	>16	>16	8–>16
	Doxycycline	8	8	<1–64
	Colistin	2	>32	2–>32
	Rifampin	8	8	<1–>128
A. baumanii (25)	Azithromycin	4	>16	<1–>128
	Doxycycline	<1	<1	<1–64
	Colistin	2	2	1–4
	Rifampin	2	>128	<1–>128

Results: High MIC values were detected against MDR- *P. aeruginosa* strains for tested antibiotics. Colistin susceptibility was 89% for these bacteria but the MIC90 value was high (>32 mg/L). Of the tested antibiotics doxycycline and colistin seemed to be active against MDR- *A. baumanii* strains while azithromycin and rifampin have high MIC values. Hundred per cent of isolates were susceptible to colistin.
Conclusion: MDR- *Pseudomonas* and *Acinetobacter* strains, which cause nosocomial infections with an increasing ratio in recent years, have limited treatment options. According to our *in vitro* study results non-traditional antibiotics such as doxycycline and colistin can be an alternative for the infections caused by MDR-*Acinetobacter* isolates but not for *Pseudomonas*.

P1222 Nosocomial infections by *P. aeruginosa* in Brazil – MYSTIC Program 2003

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Objective: To establish the susceptibility pattern of *P. aeruginosa* isolated in 20 Brazilian hospitals during the MYSTIC Program 2003.
Methods: Clinically significant *P. aeruginosa* isolates ($n = 470$) were collected from hospitalised patients in 20 hospitals in nine Brazilian cities during 2003. Only one isolate per patient was included. A central laboratory confirmed the identification and determined the minimal inhibitory concentrations (MICs) of meropenem (MEM), imipenem (IPM), ciprofloxacin (CIP), ceftazidime (CAZ), cefepime (CPM), piperacillin/tazobactam (PIP/TAZ), amikacin (AK), gentamicin (GEM), and tobramycin (TOB) using the Etest methodology. Interpretative criteria used were those described by NCCLS document M100-S13.
Results: Fifty-seven per cent were isolated from intensive care units, 27% from general wards and 11% from neutropenic patient units. Blood cultures represented 11.4% of all clinical samples. Susceptibility patterns (%) are shown in the table below:

	PIP/TAZ	AK	MEM	CAZ	CPM	IPM	TOB	GEM	CIP
Susceptible	62.8	62.5	61.1	56.9	56.1	55.8	51.7	50.6	47.5
Intermediate	–	4.2	3.9	8.3	16.9	6.4	7.8	2.8	4.2
Resistant	37.2	33.3	35.0	34.7	26.9	37.8	40.6	46.7	48.3
MIC50	32.0	4.0	1.5	4.0	8.0	3.0	1.5	4.0	2.0

Conclusions: Susceptibility was highest for PIP/TAZ, AK and MEM, although no drug presented high activity. The pattern observed suggests specific resistance mechanisms in the region, possibly with metallo-beta-lactamase presence. Also, previous editions of the program in Brazil have shown significant clonality of carbapenem resistant *P. aeruginosa* inter- and intra-centres contributing to elevated resistance rates. The use of combination therapy to treat *P. aeruginosa* infections in Brazil may be justified.

P1223 Antimicrobial susceptibility of *Bordetella bronchiseptica* strains

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Background: *Bordetella bronchiseptica* has been considered upper respiratory tract pathogen of many mammalian species (dogs, cats, rabbits, etc.) and some immunosupressed individuals. There are few studies about antibiotic pharmacodynamics against this microorganism; however empiric prescription is very common. So the aim of this work was to determine the antibiotic profile (susceptibility-resistance, MIC, and PAE) of *B. bronchiseptica* strains isolated from different mammal species.
Methodology: *B. bronchiseptica* strains from pharyngeal swabs of some domestic species (dogs, cats, donkeys, horses, rabbits, pigs) and individuals were isolated and identified by biochemical, serological and PCR (using DAL1 and DAL3 primers) assays. We employed 10 antibiotics in order to determine susceptibility-resistance (by agar diffusion method), minimal inhibitory concentration (MIC) (by double dilutions of the antibiotics in microplaques and cultured on agar) and post-antibiotic effect (PAE) by method described by Craig and Gudmundsson (1996).
Results: Forty isolates of *B. bronchiseptica* (18 from cat, 10 from dog, six from human, two from pig and donkey, and one from rabbit and horse) were identified by PCR amplifying 1200 pb band in agarose gel; biochemical test as urease, catalase, and oxidase positive and carbohydrates no-fermentation. Hundred per cent isolated strains were sensitive to amikacin and kanamycin, 87.5% to tetracycline, 82.5% to rifampicin, 72.5% to ampicillin, 55% to erythromycin, 12.5% to penicillin and nitrofurantoin; 7.5% to vancomycin and 0% to bacitracin. The results of MIC showed heterogeneous values (the amounts varied from: amikacin 0.25–100 mg/mL, ampicillin 16–112 mg/mL, tetracycline 0.05–150 mg/mL, penicillin 50–25 000 IU, kanamycin 0.125–32 mg/mL). Also the results of PAE showed different values among strains isolated from the same specie; e.g. strains isolated from cat show values from 1 h to 5 h 40 min, while strains from dog show values from 1 h 20 min to 6 h 50 min; and human strains show values from 16 h. In general, isolated strains from human > dog > cat > donkey showed resistance for more antibiotics.
Conclusions: Many factors influence of development resistance against antibiotics, such as sub-optimal concentration exposition, in order to avoid this problematic and agree with the results of the present work suggests determinate profile antibiotic for each individual independently of species and give it appropriated dosing regimens.

P1224 Postantibiotic effect of antimicrobial combinations on multiresistant *Pseudomonas aeruginosa*

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Objectives: Management of nosocomial infections caused by multidrug-resistant (MDR) isolates relies on antimicrobial combinations. Their application would be promising only if they possess an extended postantibiotic effect (PAE). The present study focused on the determination of the PAE of these combinations on *P. aeruginosa*.
Methods: A 6 log 10 inoculum of 15 isolates resistant to ceftazidime (CZ), imipenem (IM), meropenem, aztreonam, ciprofloxacin (CIP) and amikacin (AM) were *in vitro* exposed for 24 h to CZ, IM and CIP and to their interaction with AM in tubes of Mueller–Hinton broth. All beta-lactams and AM were applied at a concentration of 16 mg/L and CIP at 2 mg/L; these concentrations are equal to the mean serum levels of the applied antimicrobials. PAE was estimated only for isolates with synergy documented after 24 h of growth. At 24 h, tubes were centrifuged and the bacterial pellet was re-suspended in broth; bacterial growth was then estimated at regular time intervals. PAE was assessed as the time period requested for bacterial growth to increase more than 1 log 10. The effect of antimicrobial combinations was calculated after subtracting the PAE of the most potent single agent.
Results: Synergy at 24 h was found in nine (60%), seven (46.7%) and three (20%) isolates by the interaction of CZ and AM, of IM and AM and of CIP and AM, respectively. Mean ± SE PAE of these interactions was 7.11 ± 3.2, 14.93 ± 3.29 and 16.17 ± 7.83 h, respectively.
Conclusions: *In vitro* synergism between beta-lactams and AM or between CIP and AM is accompanied by an extended PAE providing pharmacodynamic evidence for the need of the application of these combinations for the management of infections by MDR *P. aeruginosa*.

P1225 *Achromobacter* (*Alcaligenes*) bacteraemia: a 14-year retrospective analysis at a comprehensive cancer centre in the United States

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Objective: *Alcaligenes* species may occasionally lead to serious infections in patients with an underlying malignancy. We sought to determine treatment options in cancer patients with *Alcaligenes* bloodstream infection.
Methods: Retrospective analysis of *Alcaligenes* species isolated between 26 December 1989 and 27 July 2003 was performed at M.D. Anderson Cancer Center, Houston, USA.
Results: Among 387 patients, *Alcaligenes* species was isolated from 699 culture samples. Forty-six patients (12%) were bacteraemic; 1.8 positive blood culture per patient (82 positive cultures). In 28 patients (61%) single blood culture sample grew *Alcaligenes* species; 18 (39%) had multiple positive blood cultures (11 had two (+) cultures, five patients had equal to or greater than three (+) cultures and in one patient bacteraemia remained persistent at equal to or greater than seven (+) blood cultures). Most bloodstream isolates ($n = 76$; 42 patients, 91%) were *A. xylosoxidans*; *A. denitrificans* and *A. faecalis* were other identifiable species. In 34 patients (74%) *Alcaligenes* bacteraemia was monomicrobial, whereas in nine individuals (20%) two organisms were identified, and in 6% ($n = 3$), three or more microorganisms-associated concomitant bacteraemia–fungaemia was noted. *In vitro* susceptibility was performed in 43 patient isolates, 28 antimicrobial agents were tested. The MIC90 for all *Alcaligenes* species was within susceptible range for carbapenems, piperacillin–tazobactam, ticarcillin–clavulanic acid, and trimethoprime–sulfamethoxazole. MIC50 was in non-susceptible range against fluoroquinolones (ciprofloxacin, levofloxacin), aminoglycosides, aztreonam, ampicillin–sulbactam.

Following are the yearly distribution of blood culture *Alcaligenes* isolates: [year, total isolates, blood isolates *n* (%)] 1989, 1, 0 (0%); 1990, 9, 2 (22%); 1991, 23, 6 (26%); 1992, 19, 1 (5%); 1993, 20, 2 (10%); 1994, 30, 3 (10%); 1995, 21, 3 (14%); 1996, 18, 3 (17%); 1997, 20, 4 (20%); 1998, 30, 4 (13%); 1999, 49, 4 (8%); 2000, 60, 4 (7%); 2001, 53, 6 (11%); 2002, 53, 5 (9%), and in 2003, 34 total *Alcaligenes* clinical isolates, 1 (3%) was isolated from blood culture specimen.

Conclusions: Since 1990, we observed no increase in *Alcaligenes* bloodstream infections in patients receiving cancer treatment at our institution. The susceptibility data indicates that empiric antimicrobial regimens consisting of fluoroquinolones, aminoglycosides, or monobactams may be unsuitable for cancer patients with systemic *Alcaligenes* infection.

P1226 *In vitro* susceptibility of antibiotic resistant *Escherichia coli* from urine samples to ertapenem and twelve other antibiotics

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Objectives: The purpose of this study was to examine the *in vitro* susceptibility to ertapenem and 12 other antibiotics of antibiotic resistant *E. coli* strains isolated from recent urine samples sent to the microbiology laboratory.

Methods: A total of 315 strains from three microbiology laboratories of the Community of Madrid (Spain), were collected during the year 2003. The strains were selected based on their resistance to ciprofloxacin and/or gentamicin and/or cefotaxime and/or their production of extended-spectrum beta-lactamases (ESBL). The minimum inhibitory concentration (MIC) for each antibiotic was determined using the agar dilution method following the recommendations of NCCLS. The detection of ESBL production was based on the agar diffusion technique using E-test strips of cefotaxime/cefotaxime clavulanate and ceftazidime/ceftazidime clavulanate, and cefoxitin discs.

Results: See table below:

Antibiotic	Range	MIC_{90} (mg/L)	%S	%R
Ampicillin	≤2–>16	>16	7	92.4
Cefazollin	≥1–>16	>16	64.4	26.6
Cefuroxime	≤1–>16	>16	70.8	23.5
Cefotaxime	≤0.06–>16	>16	84.1	14.6
Amoxicillin/ clavulanate	≤2/1–>32/16	32/16	57.1	16.2
Piperacillin/ tazobactam	≤1/4–>64/4	16/4	95	2
Imipenem	≤0.06–1	0.5	100	0
Ertapenem	0.008–4	0.06	100	0
Gentamicin	≤0.5–>8	>8	70.2	27
Amikacin	≤1–16	4	100	0
Fosfomycin	≤1–>128	8	97.2	2.2
Ciprofloxacin	≤0.12–>4	>4	10	89.6
CO-trimoxazole	≤0.5/9.5–>4/76	>4/76	25.7	74.3

On comparing antibiotic resistance among ESBL-producing strains ($n = 35$) and non-ESBL-producing strains ($n = 280$), statistically significant differences were obtained in the first group for resistance to ciprofloxacin ($P = 0.002$) and to gentamicin ($P = 0.011$).

Conclusions: (1) All the strains were susceptible to ertapenem, imipenem and amikacin. Ertapenem was the most active of all drugs tested (MIC90 = 0.06 mg/L). (2) The ESBL-producing strains were significantly more resistant to ciprofloxacin and to gentamicin than those that do not produce ESBL. (3) In view of its antimicrobial power and, in addition, its favourable pharmacokinetic characteristics, ertapenem may constitute a good therapeutic alternative for urinary infections caused by antibiotic resistant *E. coli*.

P1227 Susceptibility to ertapenem and other antibiotics of Amp-C producing enterobacteria isolated from urine samples

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Objectives: The purpose of this work was to study the *in vitro* activity of ertapenem and 12 other antibiotics against recent Amp-C producing enterobacteria isolated from urine samples sent to the laboratory for the diagnosis of urinary infection.

Methods: A total of 98 strains from three microbiology laboratories of the Community of Madrid (Spain), were collected during the year 2003. The type and number of microorganims was as follows: *Morganella morganii* (39), *Enterobacter cloacae* (30), *Enterobacter aerogenes* (9), *Enterobacter asburiae* (2), *Citrobacter freundii* (5), *Serratia liquefaciens* (1), *Serratia marcencens* (6), *Providencia stuartii* (6). The minimum inhibitory concentration (MIC) for each antibiotic was determined using the agar dilution method following the recommendations of NCCLS.

Results: See table below: Ertapenem always showed a MIC equal to or less than that of imipenem, with the exception of four strains of *Enterobacter cloacae*. None of the bacteria studied showed resistance to the tested carbapenems, and only nine strains of *M. morganii* showed intermediate susceptibility (MIC = 4 mg/L) to imipenem.

Antibiotic	Range	MIC_{90} (mg/L)	%S	%R
Ampicillin	>16	>16	0	100
Cefazollin	>16	>16	0	100
Cefuroxime	2–>16	>16	28.5	65.3
Cefotaxime	≤0.06–>16	>16	87.7	12.3
Amoxicillin/ clavulanate	16/8–32/16	>32/16	0	99
Piperacillin/ tazobactam	≤1/4–>64/4	16/4	92.8	4.1
Imipenem	0.12–4	2	90.8	0
Ertapenem	0.008–4	0.5	100	0
Gentamicin	≤0.5–>8	2	90.8	6.2
Amikacin	≤1–8	4	100	0
Fosfomycin	≤1–>128	>128	47	41.8
Ciprofloxacin	≤0.12–>4	>4	79.6	16.4
CO-trimoxazole	≤0.5/9.5–>4/76	>4/76	79.6	20.4

Conclusions: Ertapenem is a powerful agent against betalactamase Amp-C producing enterobacteria. These bacteria showed a MIC90 of 0.5 mg/L to ertapenem, and all the strains studied were susceptible to this antibiotic. Ertapenem was the most active of all drugs tested. Ertapenem and amikacin may constitute a good alternative for urinay infections caused by these pathogens, as well as piperacillin/tazobactam, imipenem and gentamicin that were active against more our 90% of the strains.

Viral diseases

P1228 Evaluation of measles-specific immunity in a high-risk group

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Objectives: The main task of our research was definition of specific parameters of humoral and cellular immunity of the medical staff having contacts with measles virus (MV) patients.
Methods: With this purpose the anamnesis data of 19 medical workers have been analysed. Saliva and serum specimens were collected from every subject. For extraction of human peripheral blood mononuclear cells (PBMC) the whole donors' blood was used. For evaluation measles-specific humoral immunity serum immunoglobulins G were measured using commercial test-system 'Enzygnost(R) Anti-Measles Virus/IgG' (Dade Behring Marburg GmbH, Germany). The virus-neutralising activity of serum samples was determined by plaque reduction neutralisation test (PRN) and haemagglutination inhibition test (HAIT). Moreover, we detected the ability of serum antibodies to bind with native MV proteins as well as recombinant haemagglutinin (NH) by Western-blot assay. The levels of specific IgA and IgG in saliva were defined by enzyme-linked immunosorbent assay (ELISA). We used inactivated MV and recombinant NH protein as antigens for ELISA. Measles-specific T-cell response was measured by proliferation of PBMC incubated with different measles antigens compared with control cells which were incubated with nonspecific antigen. An ELISpot assay was used to detect IFN-gamma producing cells after stimulation with measles antigen. The stimulation index was the ratio of mean spots in specific and nonspecific activated cell wells.
Results: High level of specific IgG was detected in all examined serum samples. These data had a strong correlation with virus-neutralising activity of sera. Research of saliva specimens revealed high production of specific IgA. Interestingly, that detection of specific antibodies to NH protein was observed with high virus-neutralising activity of serum in the same time. The level of measles-specific T-cell response was significantly higher after stimulation with the native virus than recombinant NH protein. Similarly, mean number of mononuclear cells producing IFN-gamma was higher in the presence of the native virus.
Conclusion: The study of anamnesis data revealed no cases of MV disease inside of examined group with high risk of the infection. The fact allows to assume that protective anti-measles immunity is due to presence of the specific IgA in saliva, high virus-neutralising activity of serum antibodies in combination with high level of cellular immunity.

P1229 Evaluation of oral fluid IgM, RT-PCR and genotyping for early diagnosis and characterisation of cases in Swiss measles outbreaks

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Introduction: In measles surveillance, the use of oral samples rather than blood may improve patient early diagnosis and epidemiological studies because of better acceptance of non-invasive samples by patients and physicians.
Objectives: To assess suitability of oral fluid as sample and of salivary IgM and PCR as methods for rapid diagnosis of measles infection in an epidemiological study.
Materials and methods: In response to clinical and/or laboratory notification of measles cases to the Swiss Federal Office of Public Health, physicians were invited to provide oral fluid samples using sponge swabs along with dates of sampling and onset of symptoms. IgM EIA adapted for oral fluid (Microimmune, UK-Brentford) and, following overnight transport of samples, nested RT-PCR (Robert Koch-Institute, Berlin) were performed. Reactive samples were genotyped using the variable part of the N gene. A novel LightCycler PCR (Viollier) was performed on samples frozen at $-80^{\circ}C$ immediately after receipt.
Results: 91 samples were collected 2–70 days after the onset of symptoms. Nineteen were IgM and nested RT-PCR negative, 55 IgM positive and nested RT-PCR positive, four IgM negative and nested RT-PCR positive, 17 IgM positive and nested RT-PCR negative. Retesting of these 17 samples by LightCycler PCR revealed 13 positive (all collected within the first week after onset of symptoms) and four negative results (two collected >2 weeks and two borderline IgM). Genotyping revealed 26 D5, 6 D7, and 12 D8 measles virus infections.
Conclusions: Oral fluid is suitable for early diagnosis of measles infection. In the first week after the onset of symptoms PCR may be superior to IgM detection. It also allows for genotyping. The detection of D5 and D8 genotypes, which are prevalent in Asia but not in Europe, suggests that genotyping may be useful for epidemiological studies. Discrepancies between nested RT-PCR and LightCycler PCR will require further analyses because of different preanalytical conditions. Oral fluid IgM may be useful in PCR-negative samples collected >2 weeks after onset of symptoms.

P1230 A measles outbreak in an ultra-orthodox partially immunised community

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Objectives: Although measles is a vaccine preventable disease, outbreaks continue to occur, particularly in unimmunised or partially immunised populations. Lately, an outbreak occurred in an ultra-orthodox community in Jerusalem. An epidemiologic investigation and a vaccination campaign were conducted.
Patients and methods: The first cases of measles were reported in the middle of March 2003. All patients were from the ultra-orthodox community. The index case was a 2-year-old child who arrived as a visitor to Israel while in the incubation phase of measles. The disease spread rapidly throughout the community during the holiday's season. Many patients and families had had minimal contact with health authorities, and were wary of any such association. Contact with the community leaders and involvement of an ultra-orthodox-managed voluntary organisation facilitated access to the population.
Results: Within 4 months 102 cases of measles were recorded, of whom 90 (88%) were unimmunised. The mean age was 8.3 years (range: 4 months to 43 years). No serious complications of the disease were observed. A large scale immunisation campaign was initiated and in the first 3 days, over 2000 infants were immunised, followed by over 6000 children who were immunised within ultra-orthodox educational institutions. Fifty per cent of those children had no previous contact with preventive health services. The last case was observed in August 2003.
Conclusions: The population and timing of the outbreak created unique problems necessitating a coordinated effort in order to interrupt measles transmission. In spite of high immunisation coverage country-wide, close surveillance of susceptible populations is essential. The social derivatives will hopefully assist the use of public health measures in the future.

P1231 Serological screening for CMV antibodies during pregnancy: possibilities to select a high-risk group for congenital infection

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Objective: Screening programs for congenital CMV infection (CCMV) are difficult to implement since this implicates routine viral culture of urine from all neonates. In this study we evalu-

ated the feasibility of a serological screening program for detecting neonates at risk for a CCMV infection.
Methods: 7140 unselected mother-infant pairs were included. In the mother a serological screening consisted of the detection of CMV-antibodies (IgG and IgM) at the first prenatal visit and at delivery (on cord blood). In the neonate CMV urine culture was performed to diagnose congenital infection.
Results: Serological screening showed evidence of past infection in 3850 women (53.9%); 3098 (43.4%) women had no antibodies in their first serum sample, and 192 (2.7%) women had both IgG and IgM antibodies when first tested during pregnancy. Seroconversion during pregnancy was detected in 44 of the seronegative women (1.4%). Forty-four CCMV infections were diagnosed (0.64%): eight in the group of past infections; 22 out of the 44 women who seroconverted during pregnancy; 14 in the group of women with a positive IgM in their first serum sample. IgM positivity on cord blood was found in 44% of the congenital infected neonates.
Conclusion: Screening at the first prenatal visit and on cord blood, defines two major risk groups for CCMV: women with seroconversion during pregnancy and women with IgM antibodies in their first prenatal serum sample (0.6 and 2.7%, respectively, of the pregnant population). In these selected groups, CMV culture should be performed in the neonate. This type of screening allows the detection of 82% of all CCMV cases. IgM detection on cord blood increases the detection rate to 91% of all congenital CMV infections.

P1232 Frequency of human papillomavirus infection among patients with chronic tonsillitis

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Objectives: Our aim was to establish the frequency of human papillomavirus (HPV) infection in the upper respiratory tract of patients with chronic tonsillitis, to identify detected viral types and to evaluate influence of some risk factors on HPV persistence in the upper respiratory tract.
Methods: The group of random selected 50 patients (31 female and 19 male, mean age, 13.64 years; range, 2–54) with chronic tonsillitis treated in the Department of Otolaryngology, Kaunas University of Medicine, was examined. Epidemiological characteristics and objective data were analysed; routine laryngological examination was performed. The biopsies of tonsils were taken during tonsillectomy and analysed for the presence of HPV DNA using the polymerase chain reaction (PCR). Viral typing using PCR was performed as described by Tucker *et al*.
Results: HPV DNA was detected in 30% (15/50) of patients. HPV 6 and 11 types were predominant (14/50, 28% of patients). High oncogenic risk HPVs (only type 18) were detected in two (1/50) patients. None were positive for HPV 16. There were no significant differences in frequency of HPV infection in the upper respiratory tract according to age and sex ($P > 0.05$). Investigated risk factors (low resistance of immunity, the inclination to diseases of respiratory system, enlargement of submandibular lymph nodes, dental caries, and smoking) were statistically significant detected in HPV positive patients ($P < 0.001$).
Conclusions: The frequency of HPV infection in the upper respiratory tract of patients with chronic tonsillitis is high; HPV 6 and 11 types are predominated. Some investigated risk factors have statistically significant influence on persistence of HPVs in the upper respiratory tract of patients with chronic tonsillitis.

P1233 Detection of human papillomaviruses using PCR and sequence analysis in routine cervical cancer screening – correlation of HPV type and cytology

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Objectives: Persistent infection with human papillomaviruses (HPV) is the main cause of cervical carcinoma. Individual HPV types are associated with different risks for the development of cervical dysplasia and carcinoma. In this study we correlated cytologic findings with the results of a highly sensitive and type specific HPV assay routinely performed in our laboratory.
Methods: HPV tests and examination of Pap smears of 1875 cervical specimens were performed in parallel or with an interval of few weeks. HPV types were determined using PCR amplification (L1 consensus primers MY09, MY11 and GP5+, GP6+ nested primers) followed by sequence analysis. HPV16 viral loads were quantified against the beta-globin gene using a HPV16 specific LightCycler PCR protocol.
Results: 932 cervical samples (49.7%) were HPV positive. Thirty-seven different HPV types were found. Women with severe cervical dysplasia most frequently carried HPV16 (62%). HPV18, HPV31, HPV33, HPV35, HPV45, HPV51, HPV52, HPV53, HPV58 HPV62, HPV66, HPV73, HPV82 and HPV11 were found with lower frequencies in these patients. In mild to moderate cervical dysplasia, in addition to HPV16 (36%) numerous HPV types were detected which so far are not classified as high risk HPV (HPV54, HPV67, HPV70, HPV83, HPV84, HPV90, CP8304). These HPV types are dot detected by the HPV test most frequently used in routine HPV testing (Hybrid-Capture 2 assay). The HPV16 viral load was significantly higher in patients with cervical dysplasia compared with patients with normal cytology.
Conclusion: Our results show that HPV types which are not detected by the most frequently used commercial hybridisation assay (HPV53, HPV62, HPV66, HPV73, HPV82) can cause severe cervical dysplasia and possibly cervical cancer. The HPV16 viral load may predict the further progression or regression of dysplastic lesions. Cytology in combination with a type specific HPV test, which detects all HPV types and allows the classification of individual risks, will produce a more reliable and effective screening for cervical cancer.

P1234 Coxsackie viral infection in patients during 1993–2002

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Objectives: The infection with coxsackieviruses is associated with different acute clinical syndromes, but recent data refer about its important role in some chronic disorders, such as juvenile diabetes mellitus, cardiomyopathies, etc. The aim of presented study was to obtain information about incidence and trends of coxsackieviral infection in the young Slovak population. This was acquired using comparison of the markers of actual and recent infection in 655 patients with different diagnoses up to 20 years of age in a 10-year interval.
Methods: Actual coxsackieviral infection was ascertained on the basis of at least fourfold increase of virus-neutralising antibodies in paired serum samples and/or detection of virus-specific IgM antibodies in virus-neutralising tests. The presence of antibodies against individual serotypes was considered as the marker of overcome infection. The results were compared according to the groups of diagnoses (A – newly diagnosed juvenile diabetes mellitus, B – myocarditis and other cardiology diagnoses, C – other diagnoses, mainly meningitis, febrile status, exanthematous disease, etc.).
Results: Actual coxsackievirus infection was confirmed in 218 (33.3%) patients (A – 34.5%, B – 36.7%, C – 22.9%). In all diagnoses the most frequent was infection with coxsackievirus B4 (CV B4 – 38.5%) followed by CV B2 (16.1%) and CV B3 (13.6%). The most prevalent serotype in 1993–1997, 1999 and 2002 was CV B4, in 1998 CV B2, in 2000 CV B5 and in 2001 CV B3. In the individual sera the antibodies were detected against 1.71–5.0 serotypes (mean); the number was lowest in A group and highest in C group. This finding seems to support the so called 'hygiene hypothesis', which stresses the role of immune experience with coxsackieviral infection in early childhood in the pathogenesis of juvenile diabetes mellitus. In all groups a decrease in mean number of viral exposures during the followed interval was observed.

Conclusions: The circulation of coxsackieviruses in our population is relatively frequent. The role of coxsackieviruses, as proved in the pathogenesis of many clinical syndromes, accentuates the need for regular monitoring of the prevalence of coxsackieviral infection in the young population.

P1235 Human enteroviruses (HEV) in the treated wastewater of the Slovak Republic: a 4-year monitoring (1999–2003)

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Background: Human enteric viruses enter the water environment through the discharge of waters contaminated with sewage. The assessment of these circulating enteroviruses gives an indication of the human exposure to the enteric viruses. They are shed in the faeces of infected individuals (clinically overt or asymptomatic).
Objective: To investigate the presence of the non-polio human enteroviruses in wastewater in different districts of the Slovak republic.
Methods: During the years 2000, 2001, 2002, 2003 samples were collected from the wastewater treatment plants from 42 localities in the Slovak republic four to five times per year and processed by the standard methods recommended by the WHO Regional Reference Laboratory (RRL) in Helsinki. Samples in volumes of 1 L were concentrated using a two-phase method of separation: polyethylene-glycol (PEG) and dextran. Two eluates were collected – from the bottom and the interphase – and both were separately treated with chloroform. The processed samples were inoculated in three cell lines: RD (rhabdomyosarcoma), Hep-2 (epidermoid carcinoma), L20B, a mouse cell line expressing the human poliovirus receptor (PVR: CD155). Two blind passages were made. When a cytopathic effect was observed the isolates were identified by the virus neutralisation test using typing pools of horse serum for typing according to Lim Benyesh-Melnick of enteroviruses. The typing pools were obtained from the National Institute of Public Health and the Environment, Bilthoven (RIVM).
Results: Year 1999: ECHO 6 was isolated in October mainly from the different districts of the Banska Bystrica region. Year 2000: Coxsackievirus B5 was isolated in October from different districts of Trnava region. Year 2001: mainly Coxsackievirus B5 and ECHO 30 were isolated from the districts of Banska Bystrica, Presov and Trnava regions. Year 2002: Coxsackievirus B3 was isolated from in the months of September and October and the districts of Bratislava, Trnava and Kosice regions. Year 2003: ECHO 6, ECHO 12, CVB 3, CVB 4 and CVB 5 were isolated in the months of June, July and August from the districts of Bratislava, Trnava, Kosice and Nitra regions.
Conclusions: Our results show the presence of different enteroviruses in treated wastewater indicating that these viruses are circulating in the population living in those areas. The study demonstrates the value of wastewater analysis for sentinel of gastrointestinal viruses in the population.

P1236 The therapeutic effects of milrinone in treatment of enterovirus 71-induced pulmonary oedema

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Objectives: To evaluate the potential therapeutic effect of milrinone, a phosphodieasterase (PDE) inhibitor, in treatment of patients with enterovirus 71 (EV71)-induced pulmonary oedema.
Methods: Twenty-four children with severe EV71-induced pulmonary oedema were enrolled, from April 1998 through June 2003, in southern Taiwan. They were divided into groups treated before and after the introduction of milrinone therapy. Aetiological diagnosis was made by viral cultures and identified by specific immunofluorescence and neutralisation tests.
Results: All 24 patients were below 5 years of age. The mortality was lower in milrinone-treated than in milrinone-untreated group (36.4 vs. 92.3%, $P = 0.005$). Sympathetic tachycardia was decreased in patients treated with milrinone compared with controls (205.7 ± 25.8 vs. 143.5 ± 17.1/min, $P = 0.004$). A marked decrease in IL-13 (76.6 ± 8.8 vs. 162.1 ± 87.5 pg/mL, $P = 0.001$) was observed in milrinone-treated patients compared with controls. There was a significant reduction in WBC ($10\,800 \pm 500$ vs. $19\,500 \pm 800$/cmm, $P = 0.009$) and platelet counts ($256\,600 \pm 44\,700$ vs. $400\,100 \pm 86\,500$/cmm, $P = 0.001$) in milrinone-treated patients compared with controls.
Conclusions: The administration of milrinone was associated with a lower mortality in patients with EV71-induced pulmonary oedema and cardiopulmonary failure. The results appear to be related to improvement in sympathetic regulation and decrease in IL-13 production. Milrinone therapy may provide a valuable therapeutic approach for this highly lethal disorder.

P1237 Detection of the aetiological predominance of norovirus in the hospital (nosocomial) gastroenteritis outbreaks in Hungary

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Objectives: Outbreaks of acute gastroenteritis associated with norovirus (Caliciviridae) cause serious health problems in hospitals. In order to identify the magnitude of the norovirus outbreaks in hospitals, including their epidemiological and clinical characteristics and the genetic variability of the detected calicivirus strains, hospital outbreaks of gastroenteritis were investigated in Hungary.
Methods: Between 1 November 1998 and 1 May 2003, 447 stool samples from reported non-bacterial and non-rotaviral hospital gastroenteritis outbreaks were tested for norovirus by reverse-transcription polymerase chain reaction (RT-PCR) followed by sequencing and enzyme immunoassay (EIA).
Results: Eighty-six (54%) of the reported 160 (data from K. Böröcz, NCE, Budapest) hospital gastroenteritis outbreaks were investigated for norovirus and 85 (99%) epidemics were positive by RT-PCR ($n = 74$) or EIA ($n = 11$). In sixty-eight (92%) of the 74 RT-PCR-positive outbreaks norovirus were confirmed by sequencing. Outbreaks occurred mainly in wards for internal disease patients (43%) and involved more than two wards (16%). Personal contact was the most common mode of transmission. Average attack rate was 20% but it was 32% (0–80%) for the hospital staff. Outbreaks lasted for 14 days (3–53 days) on average. The main symptoms were diarrhoea (87%) and vomiting (51%). Genotyping revealed that diversity and frequency of the genotypes changed over time, although the genogroup II strains predominated (97%). Outbreak strains grouped in clades Lordsdale ($n = 55$; 78% including new-variant Lordsdale virus, $n = 30$), Hawaii ($n = 5$; 7%), Wortley ($n = 3$; 4%), Birmingham ($n = 2$; 3%). Moreover a recently emerged group of recombinant strains ($n = 6$; 8%) with four different capsid types were also detected (GGIIb/Hilversum polymerase). Both epidemic clusters caused by genetically identical strains and mixed infections with genetically different strains at the same wards were observed. A distinct peak of the norovirus outbreaks ($n = 43$; 52%) was seen in the winter period of 2002/2003, associated with a new-variant Lordsdale virus.
Conclusions: In Hungary, this is the first countrywide surveillance of norovirus gastroenteritis outbreaks in hospitals. The study confirmed that noroviruses are the most frequent etiological agents preceding other enteric bacterial and viral pathogens in the hospital. These nosocomial outbreaks cause significant financial and public health problems in the country's health-care providing system.

P1238 Molecular epidemiology of astrovirus infection in Italian children with gastroenteritis

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Objectives: A 1-year study involving children suffering from acute diarrhoea was conducted from August 1999 to July 2000 in Palermo, Italy. Our goals were: to define the epidemiological role of human astrovirus (HAstV) as a cause of gastroenteritis in Italian children; to compare these illnesses with those caused by other enteric viruses; to analyse genetic correlation between our HAstV isolates and prototype strains from other geographical areas.
Methods: Stool specimens were collected from 439 children, hospitalised at the 'G. Di Cristina Children's Hospital', presenting three watery stools in a period of 24 h with a sudden onset. Routine diagnostic tests for rotavirus and bacterial pathogens were carried out on all specimens. One hundred and fifty-seven rotavirus and bacteria-negative specimens were tested for the presence of HAstV, adenovirus, and norovirus by EIA tests (Cambridge, Bioscience, Worcester, MA; Dako Diagnostics, Cambridgeshire, UK). All specimens positive for HAstV were confirmed by reverse transcription (RT)-PCR amplification and isolation on PLC/PRF/5 human hepatoma cell line. HAstV amplified ORF2 region was sequenced for genetic typing.
Results: Rotavirus was detected in 179 cases (40.7%). HAstVs were found in five cases (3.1%), adenoviruses type 40/41 in eight (5%), non-enteric adenoviruses in five (3.1%), and norovirus in nine (5.7%) (Table 1). Concurrent infection was detected in two samples. All of HAstV infected children were less than 2 years of age (median age: 13.4 months). HAstV infections occurred between March and May. The disease induced by HAstV was mild and comparable to those caused by norovirus and adenovirus type 40/41. The BLAST analysis of the ORF-2 sequences referred the single 1999 isolate and three of the four 2000 isolates to serotype 1, while the fourth was of serotype 3. The phylogenetic tree including all published HAstV sequences clustered the three 2000 type 1 isolates together (Figure 1). Analysis of the nucleotide and amino acid sequences of the capsid region determining the serotype showed high conservation within our serotype 1 isolates. Amino acid similarity was lower between our type 3 isolate and reference strains.

Table 1 Demographic and clinical findings associated with single viral infections in children hospitalized with acute gastroenteritis in Palermo, 1999–2000.

Characteristic	Type of infection				
	Rotavirus	Adenovirus	Adenovirus 40/41	Non-enteric adenovirus	Norovirus
No tested	439	157	157	157	157
No. (%) of children infected	179 (40.7)	5 (3.1)	8 (5)	5 (3.1)	9 (5.7)
No. sex male	99 (55.3)	4 (80)	3 (37.5)	2 (40)	4 (44.4)
Age (mo.)	14.7 (7.9–23.2)	13.4 (3–13.6)	6.7 (2.8–18.2)	12.2 (2–17.3)	12.2 (7.5–22.9)
Days of diarrhea	5 (3–6.5)	4 (4–4)	4 (3–5.5)	6 (3.5–7.5)	4.5 (4–6.5)
Maximum number of stools/day	6 (4–10)	6 (3–8)	5.5 (3–6)	8.5 (6–11.5)	7 (5.5–9)
Vomiting (%)	153 (8.5)	3 (60)	6 (75)	3 (60)	3 (33.3)
Days of vomiting	1.5 (1–2)	1 (0–1)	1.5 (0.5–2.5)	2.5 (1–3.5)	1 (1.5–1.5)
Fever (%)	135 (75.4)	2 (40)	3 (37.5)	3 (60)	6 (66.6)
Maximum fever (°C)	38.5 (38–39)	38.5 (38–39)	38.2 (37–39.2)	39 (38.5–39)	39.1 (37.7–39.3)
No. (%) of dehydrated children	71 (40)	2 (40)	1 (12.5)	2 (40)	1 (11.1)
Days of hospitalization	4 (4–6)	5 (4.5–5.5)	4 (3.5–4.5)	5.5 (4.5–6.5)	3.5 (3–5.5)
Mean seventy score (SD)	9 (8–11)	8 (3–9)	7 (6–9)	11	8 (6–9)

If not otherwise specified data are median and (lower and upper quartiles).

Conclusions: This is the first report of molecular characterisation of HAstV isolates in Italy; our results provide additional information about HAstV strains causing enteritis in children. For adequate prevention strategies further epidemiological studies on astroviral enteritis are needed.

P1239 Incidence of Norwalk like viruses in stool samples of patients with gastroenteritis in the Tyrol, Austria 2002/2003

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Objectives: Viral gastroenteritis is a common illness in humans with high morbidity reported worldwide. In recent years, Norwalk like Viruses (NLVs) have emerged as an important cause of viral gastroenteritis in people of all age groups and showed to be the main cause of outbreaks of gastroenteritis in institutions such as nursing homes and hospitals. Norovirus infection usually presents as acute-onset vomiting, diarrhoea and abdominal cramps. The aim of this study was to elucidate the incidence of NLVs in stool samples of patients with the clinical diagnosis gastroenteritis in the Tyrol.
Methods: From August 2002 until March 2003, 91 stool samples of patients (of all age groups) from the Austrian province Tyrol with the clinical diagnosis gastroenteritis were collected. Beside the routine diagnostic of enteropathogenic bacteria these faecal specimens were analysed for NLVs. The virus was detected by using a reverse transcription (RT)-PCR.
Results: NLVs were detected in 23 (25.27%) of 91 faecal specimens tested. Five of these NLV positive specimens were associated with an outbreak in a hotel. There was one patient with dual infection of NLVs and *Salmonella* and two patients with NLVs and *Campylobacter*. *Salmonella* were found in only 11 (12.09%) of the analysed stool samples. Other pathogens such as *Campylobacter*, EHEC (enterohemorrhagic *E. coli*), *Yersinia*, *Shigella*, Rotavirus and *Clostridium difficile* were found to a lesser percentage.
Conclusions: NLVs are very frequent agents of diarrhoea. Efforts should be made to establish a laboratory based rapid detection system for food-borne viruses because early diagnosis and immediate hygiene measures are the best way to avoid epidemic spread.

P1240 The investigation of parvovirus B19 infection in patients with haematologic disorders and chronic anaemia by using PCR and ELISA

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Objectives: Human parvovirus B19 (B19) causes a variety of disease syndromes determined by the age and haematological status of the host. In immunologically healthy hosts, B19 may cause erythema infectiosum in children, acute polyarthritis in adults, transient aplastic crisis in patients with haemolytic anaemia and has been associated with fetal death. Parvovirus B19 has a marked tropism for erythroid progenitor cells and this may lead to chronic anaemia in predisposed individuals. It was the purpose of the present study to investigate prospectively the frequency of parvovirus B19 infections with a diagnosis of haematologic disorders and chronic anaemia.
Methods: In order to determine the diagnostic use of different markers of parvovirus B19 infection, serum specimens obtained from 105 patients (85 adults, 20 children) with haematologic disorder (acute and chronic leukaemia, malignant lymphoma, haemolytic anaemia, myelodysplastic sydrome, aplastic anaemia, etc.) were tested for specific antibodies and viral DNA through the use of PCR and ELISA techniques. All patients in the study met standard criteria for chronic anaemia.
Results: Evidence of parvovirus B19 infection was found in 33/105 (31.4%) patients by demonstrating viral DNA and/or specific IgM antibody. Parvovirus B19 infections was established in three of 11 patients with chronic myeloid leukaemia, in three of 11 patients with acute myeloid leukaemia, in two of six patients with autoimmune haemolytic anaemia, in two of five patients with aplastic anaemia, in two of 11 patients with multiple myeloma, in three of eight patients with hodgking lenfoma, in five of 10 patients with non-hodgking lenfoma, in one of six patients with myelodysplastic syndrome, in three of patients with Tha-

lassaemia, in four of 11 chronic lenfositic leukaemia, in six of 19 acute lenfositic leukaemia. In four of the 33 positive patients only parvovirus B19 DNA could de detected, while nine patients tested positive for both parvovirus B19 DNA and specific IgM. In the remaining 20 positive patients only specific IgM could be detected.
Conclusion: Since no predictive paraclinical or clinical features were observed, we recommend that all cases of haematologic disorders with chronic anaemia be tested for the presence of parvovirus B19 infection. Due to the discrepancies between DNA and IgM results, the diagnostic procedures should include a search for specific DNA by PCR methods if specific IgM has been found to be negative.

P1241 The relationship between arthritis and parvovirus B19 infection

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Objectives: It has been claimed that parvovirus B19 infection may play a role in autoimmune diseases like rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). In order to evaluate the role of parvovirus B19 in aetiopathogenecity of these diseases, synovial fluids and serum samples were investigated for parvovirus B19.
Methods: Synovial fluids and serum samples were collected from 20 patients with early synovitis and 31 patients with RA at 1-month intervals. Serum samples were also collected also from 25 patients with SLE, 25 patients with osteoarthritis as diseased control group and 50 people were used as healthy control group in this study. The detection of parvovirus B19 IgM and parvovirus B19 IgG in serum samples were performed using ELISA and the detection of parvovirus B19 DNA in synovial fluid samples was performed with polymerase chain reaction (PCR).
Results: Our PCR study demonstrated that parvovirus B19 IgM, B19 IgG and B19 were present in three patients of early synovitis group. Two of these three patients were later diagnosed with RA and one was diagnosed with SLE. Parvovirus B19 DNA was detected in synovial fluids of eight patients in the RA group and parvovirus B19 IgM and parvovirus B19 IgG were found positive in four of these patients and only B19 IgG was found positive in the other four patients with RA. Both of the control groups did not exhibit parvovirus B19 IgM positivity.
Conclusions: Our results support the other studies suggesting the possible role of parvovirus B19 in aetiopathogenesis of RA at case level.

P1242 Immunodetection of parvovirus B19 virus in human plasma: appraisal of a novel fluorometric immunoassay and comparison with an enzyme immunoassay

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Objective: Human parvovirus B19 is the causative agent for erythema infectiosum or 'fifth disease' of children. Symptoms are usually mild in healthy individuals; however, symptoms can be very severe in pregnant women and in the immunocompromised. The aim of the present work is to investigate the performance characteristics of two new viral detection systems, an antigen-capture enzyme immunoassay (EIA) method to detect parvovirus B19 in human plasma and an alternative fluorescence labelling technique (FIA) for enhanced assay sensitivity.
Method: Recombinant parvovirus B19 capsid protein was used to immunise rabbits and sheep. The antibodies produced were employed in an antigen-capture enzyme immunoassay (EIA) and a fluoroimmunoassay (FIA). The EIA utilised a conventional peroxidase-labelled conjugate, while the FIA utilised a B-phycoerythrin (fluorophore) labelled conjugate. The sensitivity of the assays were evaluated using both purified recombinant parvovirus B19 particles and plasma samples of known parvovirus viraemic load as determined using a validated PCR method.
Results: Here we show that the sensitivity of the peroxidase labelled EIA is of the order of 5×10^{-7} parvovirus B19 genome equivalents per millilitre. The EIA was able to detect virus in the presence of IgG and IgM antibodies and no false-positive results were obtained. Also we show that the EIA and FIA methods are similar in terms of sensitivity when directed toward the detection of purified recombinant parvovirus capsid protein (VP2).
Conclusion: EIA and FIA methods as presented are broadly equivalent in terms of specificity towards the parvovirus B19 antigen VP2 capsid protein. Both systems show potential utility in the screening of blood products for the presence of parvovirus B19 antigen.

P1243 Crimean Congo haemorrhagic fever (CCHF), the main aetiological cause of viral haemorrhagic fevers in the last 4 years (2000–2003) in Iran

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Objectives: CCHF virus is from Bunyaviridae family and *Nairovirus* genus and causes severe haemorrhagic symptoms in man. As this disease has been observed in many parts of Iran and epidemiological studies are useful for its control, in this study with collaboration of the Pasteur Institute of Dakar (WHO reference centre), the situation and the incidence rate of the disease in the country in recent years have been analysed.
Methods: Serum samples from 597 CCHF suspected patients have been collected from different provinces of Iran and transferred to the laboratory of Arboviruses of the Pasteur Institute of Iran. Samples have been analysed by specific ELISA method for IgM and IgG detection against CCHF, Yellow Fever, Rift Valley Fever, Dengue 2 and also by RT-PCR technique for detection of the CCHF virus genome.
Results: By using the ELISA method, 227 patients had IgM against CCHF virus (confirmed cases) and 215 had IgG. By the RT-PCR method, 34 patients were positive. Most cases of the disease were from the south-east of Iran (the Sistan-Baluchestan province). The number of suspected, confirmed and death cases according to year are as follows: 2000 (54, 20, 4), 2001 (167, 61, 7), 2002 (247, 100, 8) and January to 4 October 2003 (132, 46, 5). All confirmed cases had fever, haemorrhage and thrombocytopenia. About 79.3% were male and 20.7% female. Farmers and butchers formed 32.7% of the positive cases. About 40.1% of the confirmed cases were between 16 and 30 years. Between the confirmed cases, 43.2% had previous contact with domestic animals and 3.9% had contact with ticks. The serum samples were negative for the other VHFs.
Conclusion: CCHF is the most important haemorrhagic fever in Iran. This disease occurs more frequently in the south-east part of the country and this fact emphasises the entrance of the disease from this region. From the obtained results, RT-PCR technique together with ELISA method is the best way of detection of the disease. RT-PCR positive results are less numerous because the viraemia period is short in humans. In the first years of the survey, positive cases of the disease showed an increasing rate and this demonstrates a greater incidence of the disease, a progression in the methods of the diagnosis and a development of the skills of the laboratory staff. The decrease in the rate of the disease this year can be due to a development of the knowledge for the prophylaxis of the disease or a decrease in the proliferation and activity of the ticks.

P1244 Crimean-Congo haemorrhagic fever in Sistan and Baluchestan province of Iran, a case–control study

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Objectives: Since summer 1999 several cases of Crimean-Congo hemorrhagic fever have been reported from different parts of Iran. The main objectives of this research were determination of the most important ways and patterns of transmission, and epidemiologic characteristics of this disease.
Design: In this population based case–control study, 24 patients from Zabol and Zahedan Districts in the Sistan and Baluchestan province, reported to the Center for Disease Control of Iran, have been compared with 300 controls. The controls were sampled through 'probability proportional to size cluster sampling' method from the general population of the same districts. The following variables were checked: age, sex, living environment (rural vs. urban), education years, job, past history of tick bite, contact history with livestock, history of livestock slaughtering, presence of an animal place at home, history of keeping of livestock in house.
Results: Variables, which increased the chance of disease, include: history of slaughtering (OR = 7.57, CI: 2.21–25.91), high-risk occupations (OR = 4.97, CI: 0.97–25.43), history of tick bite (OR = 105.89, CI: 9.32–1202.44), above 40 years of age (OR = 7.32, CI: 1.06–50.26).
Conclusion: The results of this study confirm that the scheme of risk factors and risk groups of Crimean-Congo hemorrhagic fever in Iran do not differ substantially from the other parts of the world. Even taking care of livestock for a short period at home can increase the chance for CCHF.

P1245 Detection of IgG and IgM antibodies vs. West Nile virus

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Introduction: West Nile virus (WNV) is an arbovirus. Arboviruses are zoonotic and transmitted through arthropods like mosquitoes. In recent years, in the temperate regions of Europe and North America, WNV is a threat to public health. Twenty per cent of the infected people will proceed to develop West Nile fever which portrays mild symptoms of fever, muscle aches, head aches and occasionally the appearance of a skin rash on the trunk of the body. There may also be a swelling of the lymph glands. These particular symptoms last a few days. From the individuals who develop a severe disease <1% will develop encephalitis or meningitis. The aim of this study was the detection of IgG and IgM antibodies vs. WNV in the serum of patients with meningoencephalitis and in the serum of outpatients with no symptoms of CNS.
Material and methods: 55 patients with meningoencephalitis who were hospitalised in various hospitals of Attica comprised group A (ages ranging from 18 to 67 years). A total of 133 outpatients from our hospital comprised group B (ages ranging from 16 to 78 years). The ELISA method was used to detect the presence of antibodies in the serum.
Results: No patient of group A had positive IgG or IgM antibodies but two patients of group B, one male and one female with respective ages of 42 and 53 years, showed the presence of IgG positive antibodies and IgM negative antibodies. Both individuals reported having flu-like symptoms consisting of a low fever, muscle pains and headaches 3 and 4 months before the detection of antibodies.
Conclusions: We emphasise that only IgG antibodies were detected in the group B (patients with no symptoms of CNS).We conclude that arboviruses exist in the Greek vicinity but we have no evidence from the groups we tested that these arboviruses developed meningoencephalitis.

P1246 Comparison of tick-borne encephalitis incidence in the Czech Republic under extreme weather conditions in 2001–2003

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Objectives: Tick-borne encephalitis (TBE) is very sensitive to climatic conditions. The aim of our analyses is to demonstrate the direct effect of different extreme weather conditions prevailing in three following years on the incidence of TBE human cases. These data are compared also with the activity of *Ixodes ricinus*.
Methods: TBE data incidence is taken out from EPIDAT database (National Institute of Public Health, Prague); TBE is the notifiable disease in the Czech Republic since 1951. Weather conditions in the period under study are based on database of Czech Hydrometeorological Institute (Prague) and are evaluated by standard methods of state meteorological service. Activity of *I. ricinus* was monitored by all seasonal weekly flagging of ticks on experimental plots (measuring 600 m^2).
Results: TBE incidence sharply arose in the beginning of 1990 and the high values are observed (with some fluctuation) till now due to the climate modification, as it was demonstrated in previous communications. The situation was similar in spring/summer seasons in the years under study (as for TBE incidence, weather conditions and tick activity). All three summer/autumn seasons were quite different. Oversized precipitations in 2001 caused high tick activity and thus second peak of TBE incidence approaching nearly spring/summer value. Heavy long-lasting rains in August 2002 (resulting in catastrophic floods) brought extremely wet weather conditions but influenced TBE morbidity negatively thanks mainly to decreased human outdoor activities. Very warm and dry weather (from August to November) in 2003 depressed the TBE incidence to minimal values. The changes in TBE vector *I. ricinus* activity were very similar, thus explaining the way of the weather influences on TBE incidence.
Conclusions: The presented comparison contributes to the problem of climate change influence on vector-borne diseases which is intensively studied in the WHO/EC Project Climate Change and Adaptation Strategies for Human Health in Europe. Moreover the results could be used for the prediction of TBE risk assessment according to weather conditions.

P1247 Tick-borne encephalitis risk forecast based on climate and microclimate factors affecting the activity of vector *Ixodes ricinus* tick

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Objectives: The aim is to solve the following problems: (1) Whether the relationship between the weather and tick behaviour is solid enough to be used for the prediction of tick activity, and thus for the prediction of risks of TBE infection. (2) Whether macrometeorological data provided by standard meteorological networks can be used to estimate tick activity. The study includes an examination of the tightness of relationships between the so-called macro-scale weather, as presented by standard meteorological stations, and authentic microclimate of typical forest ecosystems containing ticks and TBE virus.
Methods: Observations were realised in the south-eastern periphery of Prague, which is the site of Czech Hydrometeorological Institute Observatory (Prague-Libus), and where the experimental plots for monitoring were established in relevant type of forest growth (Querceto-carpinetum). *I. ricinus* activity was investigated by the flagging method on three plots (200 m^2 each) in weekly intervals. The instruments for micrometeorological observations were installed between the experimental plots. Meteorological elements were measured (every 30 min) in continuous 24-h measurements during 10 selected days according to different synoptic situation. Macrometeorological data were used from the CHMI meteorological observatory. Simple and multiple linear regression

and quadratic regression were used to test the relation between the weather and *I. ricinus* activity.
Results: Eight models of the relationships were tested, of which four were single-parametric and four were double-parametric. Double quadratic regression provided far better results. The relationship between daily *I. ricinus* activity and weather is characterised by distinct determinability and can be described using two- to three-parameter models.
Conclusions: A very high concordance in the trends of daily patterns of meteorological elements obtained from a standard weather station and of the microclimate of the forest containing ticks and TBE virus suggests that a warning system can be created actually evaluating and predicting the risk of *I. ricinus* tick attack and thus TBE infection based on the use of macrometeorological data as a predictor.

P1248 A qualitative detection of tick-borne encephalitis in clinical samples by real-time PCR

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Diagnosis of tick-borne encephalitis (TBE) in patients is based on clinical findings, cerebrospinal fluid (CSF) analysis and findings of specific IgM and IgG antibodies in serum. In 15–20% of patients, diagnosis is possible only from second sample of serum 7–14 days after the onset of the second phase of the disease. Therefore, there is a need of fast, sensitive and accurate method for routine diagnostic of TBE in human samples. We have adapted our existing TBEV reverse transcription PCR (RT-PCR) detection system to the one-step qualitative real-time RT-PCR. The method is based on the detection of a nuclear acid amplification during exponential phase of PCR when the reaction reaches its optimal course. In our study we have worked with the LighCycler instrument (Roche Diagnostic, UK). As a detection format, DNA binding dye SYBR Green I have been used. Specificity and sensitivity of amplification reactions were enhanced by combining amplification with a melting curve analysis. The system was established and validated using viral RNA extracted from brain tissue of suckling mice (BALB/c) infected with TBEV (strain Hypr). In order to evaluate real-time RT-PCR for routine diagnostic purposes, we have applied the assay to CSF or serum samples from patients with early diagnosis of TBE (onset of fever, headache and neurological symptoms less than 3 days before admission to hospital and lumbal punction). Diagnosis of TBE was based on typical clinical picture and serological findings in serum sample (IgM over 150 WIEU, IgG over 150 WIEU). Of the 70 clinical samples investigated for TBEV RNA, none was tested positive. To avoid false negative results we have elaborated a positive control. We have spiked a negative patient CSF sample with 10-fold dilutions of viral RNA extracted from brain tissue of infected suckling mouse. Although the method was sensitive to detect 1 PFU in a reaction volume of microlitre of artificially infected CSFs, it fails to detect viral RNA in samples from patients with serologically proven TBE. The result indicates that virus load decreases with the onset of serum antibodies and cannot be regularly detected in CSF by RT-PCR. The method is not suitable for routine diagnosis of TBEV in patients with detectable antibodies and the suitability for detection in patients without serum antibodies should be evaluated.

P1249 Changes in diagnostics and treatment of tick-borne encephalitis

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Objectives: The aim of our retrospective study is to evaluate changes in anamnestic data, diagnostic schedule, treatment and outcomes of tick-borne encephalitis (TBE) that occurred in the region of West Bohemia in the last 10 years.
Methods: We compared two sets of patients who suffered from TBE in 1994, and in 2003, respectively. Set A consists of 67 patients median age 37 years, range 1.5–77). Set B consists of 63 patients (median age 40, range 6–74). Analysis of anamnestic data, clinical features, results of laboratory investigation and serological methods – complement fixation reaction (CFR), HIT, VNT-IgM were used in 1994, and ELISA IgM and IgG were used in 2003.
Results: In the history of the illness, we noticed tick bite in 72 and 42% ($P < 0.05$), and a two-phase course in 83 and 94%, respectively. The patients of both sets were clinically presented with signs of meningitis 7.5 and 9.5%, meningoencephalitis 19.4 and 47.7% ($P < 0.05$), encephalitis 68.6 and 41.2% ($P < 0.05$) and encephalomyelitis 4.5 and 1.6%. Investigation of cerebrospinal fluid (CSF) was done in 82 and 71% ($P < 0.05$). Diagnoses were confirmed serologically in blood by CFR in set A. The results were received in 8–43 days. The blood and CSF were serologically investigated by the ELISA test; results were available in 1–5 days in set B. The treatment was supportive; corticosteroids were indicated in 66 and 63%, antibiotics in 48 and 4.8%($P < 0.05$). The lengths of stay in hospital were 12.8 and 8.6 days ($P < 0.05$). Two patients died (fatality rate 3%) and six (9%) were left with sequelae in 1994. Twenty-one patients (23%) were left with sequelae in 2003. The incidence of TBE is the same in the two sets, the diagnosis is confirmed earlier (3 days in the average), the number of CSF investigations decreased, the length of stay is shorter, and treatment with antibiotics was used 10 times less frequently.
Conclusion: The timely confirmation of diagnosis by the ELISA serological test influenced subsequent investigation and treatment, and has limited the use of antibiotics. No specific treatment for TBE has been available until now. Be vaccinated, do not become ill!

P1250 Severe courses of tick-borne encephalitis in Ostrava, 1999–2003

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Objectives: We investigated occurrence of severe as far as fatal courses of tick-borne encephalitis in patients hospitalised at the Department of Infectious Diseases, University Hospital in Ostrava, during the last 5 years.
Methods: Tick-borne encephalitis in 96 hospitalised patients at the age of 4–71 years was proved in 1999–2003. The aim of our investigation was to determine the rate of severe or fatal courses of the disease.
Results: Serious course of tick-borne encephalitis with necessity of intensive care was observed in 27 from 96 patients (28%). Five of them (5%) with bulbar form of encephalitis required assisted ventilation. One patient cured without sequelae, the other one progressed to the persistent paresis of the right upper extremity. Three patients (3%) with long-term artificial ventilation died. A 62-year-old male and a 71-year-old female died of complicated mycotic sepsis and a 30-year-old male died because of acute mediastinitis following iatrogenic damage during puncture tracheostomy.
Conclusion: Severe courses of tick-borne encephalitis represent almost one-third of cases in our group of 96 patients. However, fatal courses are not often, they were proved in three patients with bulbar form and necessity of long-term artificial ventilation. Profylactic vaccination is widely recommended, especially in adults from endemic regions.

P1251 Purification and analysis of the structure of prion aggregates from yeast *Saccharomyces cerevisiae*

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Objectives: Prions cause several incurable infectious diseases in mammals. They represent a novel type of infectious agent – misfolded homogeneous protein aggregates, which are able to cata-

lyse conformational changes of normal type protein. To investigate prion phenomenon we use yeast *Saccharomyces cerevisiae*, which contain several proteins with prion properties. The best studied of them is Sup35 prion protein. *In vitro*, purified Sup35 can form amyloid-like fibres, which was shown to be infectious after introducing into yeast cells. Moreover, in the prion state Sup35 is found in aggregated form in yeast cells, but the structure of these aggregates is unknown. Here, we present a novel method for purification of prion aggregates from yeast cells. Using it, we investigate structural and biochemical properties of yeast prions.
Methods: The method developed is based on our finding of conditions that can distinguish prions from other cellular aggregates. The key point of purifying strategy is the stability of Sup35 prion polymers in sodium dodecyl sulphate (SDS), in contrast to other protein complexes of yeast lysates. After strong SDS treatment we used ultracentrifugation or electrophoretic approach to obtain high-purified prion material. Furthermore, we used high-resolution electron microscopy to reveal the detailed structure of purified prion polymers.
Results: This approach allowed us to show that the Sup35 prion aggregates *in vivo* represent agglomerations of relatively small prion polymers with amyloid-like properties. We determined the size of polymers; this size is characteristic of a given prion variant and differs between the variants. We showed that purified prion polymers are able to convert non-prion Sup35 molecules into SDS-resistant prion aggregates in yeast lysates, which means that these polymers are infectious. We studied the effects of some prion-curing factors (such as chaperones and other chemicals) on the size of prion polymers. Finally, using electron microscopy, we obtained high-resolution pictures of prion polymers and confirmed amyloid-like structure of these polymers.
Conclusion: This work represents the first attempt to purify prion aggregates from yeast cells and to analyse their structure. The method developed may have wider application for the analysis of other prion and amyloid phenomena both in lower and in higher eukaryotic organisms.

Diagnostic methods - II

P1252 Evaluation of the VCAT3 neisseria selective medium

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Aim: To evaluate the sensitivity and specificity of the VCAT3 neisseria selective medium manufactured by BioMerieux, France.
Materials and methods: A total of 1161 randomly selected samples taken by swab from the throat of patients with upper respiratory infections were plated on both VCAT3 medium and home-made chocolate agar plates supplemented with 25 mg/L vancomycin. In another experiment a 103 CFU/mL suspension of *Neisseria meningitidis* control strain/ATCC 122004/ was added to 50 throat samples/swabs soaked in 2 mL of physiological saline/respectively at a 1:1 ratio and plated (mikrol) on the same media. All the inoculated media were incubated at 35°C for 48 h in 5% CO_2 atmosphere. The plates were read twice: after 24 and 48 h of incubation. Isolated *Neisseria* strains were identified by the API NH test (BioMerieux).
Results: Growth on the VCAT3 media could be properly evaluated after 48 h of incubation; there was no growth or only pin-point colonies of *Neisseria* were seen after 24 h of incubation. Of the 1161 throat samples, five yielded *N. meningitidis* on VCTA3 agar and three on the home-made chocolate agar plates. The *N. meningitidis* control strain could be recovered from all the 50 samples on VCTA3 agar. VCTA3 proved highly selective: 47 of the 50 samples grew exclusively *N. meningitidis* and on only three of the plates grew some colonies of additional bacteria. The sensitivity of the VCTA3 agar was also tested: *N. meningitides* (ATCC 122004) was suspended in physiological saline and 10 μl of a 5×100 CFU/mL suspension was plated on the media. All the VCTA3 agar plates yielded three to 20 colonies of *Neisseria*. Colonies of *N. meningitidis* were characteristic and easy to recognise on VCTA3 agar.
Conclusions: The selectivity of VCTA3 agar proved excellent; the medium is well-suited for the isolation of *N. meningitidis* from specimens containing large numbers of various bacteria. The sensitivity of the medium is also appropriate. Furthermore, since colonies of *N. meningitidis* are typical on VCTA3 agar plates the use of the medium can be recommended for clinical bacteriology laboratories.

P1253 A rapid antigenic test combined with a sore throat score vs. culture in group A *Streptococcal pharyngitis*

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Objective: To establish the sensitivity and specificity of three diagnostic approaches in Group A *Streptococcal pharyngitis*: a rapid antigen test, a sore throat score and a combination of these two vs. culture.
Methods: This study included 91 patients hospitalised between 15 May and 1 December 2003 in Infectious Disease Hospital from Lasi, Romania. Two pharyngeal swabs were collected from each patient, one for rapid antigen test (One Step Strep A Test Cassette – AccuBioTech – a two side sandwich immunoassay) and the other for culture. All beta haemolytic streptococci were identified as group A with a latex-agglutination kit (Slidex Strepto A – BioMerieux). The sore throat score had five criteria: age, fever, absence of cough, tender anterior cervical nodes, tonsillar swelling or exudates. The score range is −1 to 5. In this study we included only patients with no antibiotic therapy prior to examination and with no other infectious diseases.
Results: Of the 91 patients, 30 (32.97%) had a positive result for group A streptococci (culture plus latex agglutination – considered gold standard). Rapid antigen test had two false positive and one false negative results. This test had a sensitivity of 96.67% (95%CI: 82.78–99.92%) and a specificity of 96.72% (95% CI: 88.65–99.6%). Score higher or equal to 3 had a sensitivity of 51.61% (95% CI: 33.06–69.85%) and a specificity of 60% (95%CI: 46.54–72.44%). All 10 patients with score 0 had negative results for rapid strep and culture. If we add results from rapid antigen testing to all patients with score higher or equal to 3 we will not miss any positive result (95%CI: 69.15–100%) and will have a good specificity: 93.33%(95%CI: 68.05–99.83%).
Conclusions: The rapid antigen test we used had very good sensitivity and specificity. The patients with score 0 should be excluded from further testing. The rapid strep test performed on all patients with score higher than 0 will increase sensitivity and specificity of the score. Even if we cannot exclude culture, this diagnosis approach may give good results on a first visit of a patient for sore throat.

P1254 Comparison of five media for detection of group B Streptococci in vaginal/rectal swabs from pregnant women

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Objectives: Routine screening of pregnant women for group B Streptococci (GBS) rather than risk-based approach is now recommended. We evaluated five media for the detection of GBS in pregnant women 35–367 weeks gestation.
Methods: Vaginal/rectal swabs transported in Amies media (Starplex Scientific, Canada. In the laboratory, the swabs were transferred to tubes containing 0.8 mL sterile saline and vortexed. One hundred microlitres of the bacterial suspension was used to inoculate each of the following media, CNA agar with 5% sheep blood (Oxoid Canada), selective Todd Hewitt broth with 5% sheep blood (THB, Oxoid Canada), LEN GBS agar (GBSA, Northeastern Laboratory Services, Winslow, ME), NEL GBS broth (GBSB), and Instant Granada broth (IGB, Biomedics, Madrid, Spain). CNA, GBSB, GBSA, and IGB were examined after 24 and 48 h of incubation. Development of orange to red colouration in GBSA, GBSB, and IGB was considered to be an indication of GBS growth. GBSB, IGB if negative and THB were subcultured onto CNA after 24 h incubation.
Results: Specimens from 405 women were processed, 97 (24%) of these were found to be positive for GBS. Seven (7.2%) of 97 GBS were non-haemolytic and non-pigment producing and were not detected in GBSB, GBSA, and IGB. Detection of GBS after 24 and 48 h incubation was achieved in 79 (81.4%) and 80 (82.6%) specimens, respectively, by CAN, 84 (86.6%) and 86 (88.7%) specimens by GBSA, 82 (84.5%) and 86 (88.7%) specimens by GBSB and 86 (88.7%) and 88 (90.7%) specimens by IGB. Ninety-one (93.8%) GBS were isolated from IGB subcultures and 90 from GBSB and IGB subcultures.
Conclusions: All media, except CNA performed well. THB subcultures had the least overgrowth with commensal organisms. Broth media performed better than plate media. For most strains, results from GBSA, GBSB, IGB were available earlier than those of THB. However, more GBS were identified on subcultures from GBSB and IGB than could be identified in these media after 48 h incubation. Consequently turn-around time for broth media was the same.

P1255 Usefulness of fatty acid analysis for identification of nutritionally variant streptococci

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Objectives: Nutritionally variant streptococci (NVS) were described as a major causative agent of septicaemia in neutropenic cancer patients and accounted for serious cases of infective endocarditis. In clinical laboratories, the identification of NVS is often dependent upon their phenotypic characterisation. An overlap in physiologic characteristics between NVS and other Gram-positive catalase-negative cocci is known.
Methods: Our study examined, if it is possible to confirm preliminary phenotypical identification as NVS by fatty acid analysis based on whole cell fatty acid methyl esters (MIS Sherlock, MIDI, Inc., USA). Two years of study on Gram-positive catalase-negative cocci recovered from blood cultures yielded nine isolates of NVS. According to the analysis of partial 16S rRNA gene sequence, NVS-isolates were identified as *Granulicatella adiacens* (7) and *Abiotrophia defectiva* (2). To confirm correct interpretation of fatty acid results, NVS reference strains and 'viridans streptococci' isolates, which were identified as *Streptococcus* spp. and phenotypically resembled NVS, were also included in the study. The obtained fatty acid compositions of our isolates were also compared with the phenotypically similar *Gemella morbillorum*.
Results: All *Streptococcus* spp. significantly differed from other strains tested by lower amount of both C16: 1w9c and C18: 1w9c. In comparison with other NVS, only *G. elegans* showed unique fatty acid composition, the amount of C18: 2w6,9c was almost three times higher and C16: 1w9c was not detected at all. For further differentiation of *A. defectiva*, *G. adiacens* and *G. morbillorum*, three fatty acids were chosen – C16: 0, C18: 1w9c and C18: 2w6,9c. Unlike *G. adiacens* and *G. morbillorum*, the amount of C16: 0 lower than 30% and simultaneously the amount of C18: 1w9c higher than 20% were detected in *A. defectiva*. *G. adiacens* differed from *G. morbillorum* by the amount of C18: 2w6,9c being lower than 3%.
Conclusions: (1) The fatty acid composition of phenotypically similar *Streptococcus* spp. differs significantly from all tested NVS. (2) Fatty acid analysis could be an useful tool for the discrimination of *G. elegans* from *G. adiacens* and *A. defectiva*. (3) In case of failure of satelitism behaviour, the found differences in fatty acid composition could help to distinguish *G. morbillorum* from NVS.
Acknowledgement: Our work was supported by the Ministry of Health of the Czech Republic, Id.code: MZO/ZA/00538.

P1256 Evaluation of the SM ID 2 medium: comparison to the SM ID, BBL CHROMagar *Salmonella* and *Hektoën* media

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Objective: The aim of this study was to evaluate the performance of a new formula of SM ID (bioMérieux) medium, SM ID 2, particularly in terms of the specificity and sensitivity to detect various *Salmonella choleraesuis* species serotypes. The performance of the SM ID 2 medium was compared with three other media: SM ID, BBL CHROMagar *Salmonella* (Becton Dickinson) and *Hektoën* (bioMérieux).
Methods: The study consisted of testing 260 samples: 15 diarrhoea stool specimen, 171 stool specimen for bacteriological and parasitological standard examinations, 45 rectal swabs, and 29 artificially contaminated non-diarrhoea stool specimen.
Results: Seventeen non-artificially contaminated samples contained one *Salmonella* strain. With respect to sensitivity, the SM ID 2 medium is significantly more sensitive in detecting *Salmonella* species than the BBL CHROMagar *Salmonella* medium at 24 and 48 h. This medium is also more sensitive than the SMID medium at 24 and 48 h. The sensitivity of the SM ID 2 and *Hektoën* media are identical. The specificity of the SM ID 2 medium is consistently greater than 95%. Statistically, the SM ID 2 medium shows specificity significantly higher at 24 h than both the SM ID and *Hektoën* media. This difference is not present at 48 h. The specificity of the SM ID 2 medium is lower than that of the BBL CHROMagar *Salmonella* medium at 24 h but equivalent at 48 h. The difference is not statistically significant. In terms of legibility, the data are in accordance with the way the laboratory technicians are able to easily distinguish the *Salmonella* colonies on the SM ID 2 medium from the annex flora present.
Conclusion: The SM ID 2 medium is a chromogenic medium effectively adapted for routine laboratory use for the detection of *Salmonella* in stools and rectal samples. This study demonstrates better legibility performance compared with SM ID, as well as fewer false positives, which results in saving of both time and costs.

P1257 *Shewanella* species: infections in Denmark and phenotypic characterisation

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Objectives: Human infections with *Shewanella* species are rare, especially in countries with a temperate climate. We present seven case reports of typical *Shewanella* infections from Denmark and results of conventional phenotypic characterisation and antibiotic susceptibility testing on 179 Danish isolates.
Methods: Clinical records from five patients with *Shewanella* algae and two patients with *Shewanella putrefaciens* infections were

obtained. Phenotypic characterisation was made by standard methods and susceptibility testing by direct agar diffusion with Rosco Neo-Sensitabs (R).
Results: Two cases of bacteraemia, one wound abscess and two ear infections with *S. algae* are presented; in four of these cases *S. algae* was found in pure culture. All patients were treated with antibiotics specific for *S. algae*. *S. putrefaciens* was found in mixed culture in a drainage fluid and in a foot ulcer, and was not treated specifically. *S. algae* was able to grow at 42°C and in 6% NaCl, to reduce nitrite and was resistant to colistin (150 μg) in contrast to *S. putrefaciens*.
Conclusion: Differentiation between *S. algae* and *S. putrefaciens* is possible using conventional phenotypic tests. This differentiation is important because the two species have different pathogenic potentials in humans.

P1258 Simple sample preparation methods to detect Shiga-toxin-producing *E. coli* from human stools by Duopath Verotoxin

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Background: Haemorrhagic colitis and haemolytic uraemic syndrome can be caused by Shiga toxin-producing *Escherichia coli* (STEC) O157 and non-O157 STEC. Since the latter serotypes ferment sorbitol and lack other phenotypic markers, they cannot be recognised on agar plates. The Duopath Verotoxin (DV, Merck KGaA) is a new easy-to-use immunochromatographic (lateral flow) test for detecting both Stx1 and Stx2 individually on the same device. Its performance has been described recently (Park, et al., 2003; J. Clin. Microbiol. 41:2650–53). In this study, stool samples were streaked on SMAC agar and incubated overnight after which a colony sweep was harvested from the plates. The swab was suspended in distilled water containing polymixin B and after short incubation, the mixture was delivered to the sample port of DV. Both DV and Premier EHEC (Meridian Bioscience) detected all 12 fresh positive STEC (6 O157:H7 and 6 non-O157) and agreed 100% with 248 negative specimens.
Objectives: The application of DV directly from stools and overnight broth performed poorly. This study evaluated a two-step broth enrichment (24–26 h) method before applying to DV device.
Methods: As a preliminary study, 19 STEC stool samples were tested by inoculating into EHEC Diretmedium (Heipha, Germany) and/or EC broth. Overnight incubation was followed by a short post-enrichment step (4 h) in a new modified CAYE broth (Merck,KGaA). This broth was supplemented with Carbadox, which is an inducer for Stx production.
Results: All samples enriched by this method were positive when compared with the commercial ELISA test. With samples of high optical density readings, the DV correlated well yielding strong lines of signal. In most cases, the Carbadax was found to enhance the appearance of stronger signal on DV.
Conclusions: We introduced two sample preparation methods for Duopath Verotoxin, which are simple to perform and easy to interpret. However, an extended study is in order to confirm the efficacy of two-step broth sample preparation method. The DV is capable of providing a turnaround time of 24–26 h and has a great potential for clinical applications.

P1259 The biological characterisation of new phage types of *Salmonella enterica* serovar Enteritidis

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Objectives: *Salmonella enterica* serovar Enteritidis is an important cause of food-borne infections in many countries around the world. The object of this study was to characterise the chosen virulence factors (surface hydrophobicity, motility, biofilm formation) of 52 *S. enteritidis* isolates representing three new phage types identified in the Slovak Republic during the 2003.
Methods: The phage types were identified according to Ward et al. (1987). The hydrophobicity was determined on the basis of bacterial adherence to hydrocarbon-xylene (BATH) and salt aggregation test with ammonium sulphate. The assay of motility was performed on the semisolid agar medium (0.35%). The biofilm-forming abilities of strains were examined in the tube test after cultivation in a starvation medium.
Results: Fifteen strains belonged to the phage type (PT) 9a (28.8%), 26 to the PT 13a (50.0%) and 11 strains were PT 25. The results of adhesion of strains to xylene revealed a higher adhesion of strains of both PTs 9a and 25 in comparison with PT 13a. Adhesion was found to be high in 12 strains of PT 9a (80.0%) and in nine strains of PT 25 (81.9%). Motility of strains of all PTs was high. The biofilm formation after cultivation of strains in a starvation medium refer to their high virulent potential. The biofilm was formed by all strains except for two strains of both PTs 13a and 25 and one strain of PT 9a.
Conclusions: The identification of new phage types can include the differences in their reservoirs and in the distribution of contaminated food as well as in their virulence properties. The presented findings refer to the pathogenic potential of new phage types of *S. enteritidis* strains.

P1260 Phage typing of *Salmonella enterica* serovar Typhimurium isolated from humans in the Slovak Republic, 2000-2003

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Objectives: *Salmonella enterica* serovar Typhimurium is a common cause of salmonellosis among humans and animals in many countries. In contrast to countries of West Europe, the participation of this serovar in aetiology of salmonellosis in the Slovak Republic (SR) is low and epidemiologically of little significance. In our human population, serovar Enteritidis still predominates. The control of outbreaks and management of cases is greatly facilitated by the rapid isolation and identification of the responsible microorganism. Phage typing has become the reference method for the primary subdivision of strains of serovar Typhimurium for epidemiological investigations.
Methods: Two hundred and fifty-eight strains of human origin that occurred as the sporadic cases during the years 2000–2003 in the SR were examined. The strains were phage typed according to the method of Anderson et al. (1977). All strains were also tested for susceptibility to 10 antibiotics by a disc diffusion method.
Results: Seventeen phage types (PTs) were detected among the 258 isolates. The most common PT was found to be DT104 (22.9%), followed by PTs DT41, DT68, DT193, DT20a, DT6, DT12a, DT67, DT36, DT37, DT14, DT99, DT120, DT125. Ten strains were classified as react-but-do-not-conform strains (RDNC) and 97 strains were not typeable. Of DT104 isolate, 32 strains (54.2%) with characteristic resistance to ampicillin (A), chloramphenicol (C), streptomycin (S), sulphisoxazole (Su) and tetracycline (T) were found. The strains of other PTs were also resistant to antibiotics in different range.
Conclusions: The results of this study demonstrate that the multidrug-resistant DT104 strains have wide distribution in humans in the SR. With regard to this, it is important to pay attention to detection and further examination of multidrug-resistant strains of serovar Typhimurium of various phage types.

P1261 Comparison of culture methods for isolation and enumeration of *Legionella* species from cooling tower water samples

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Background and objectives: Cooling towers have been repeatedly implicated as source of *Legionella* infections in humans. The water in these cooling towers contains high numbers of contaminating bacteria. Culture of *Legionella* species from cooling tower water is

therefore difficult, and widely different results are found using different culture methods, both with regard to the number of positive samples and the number of colony forming units in positive samples. Setting standards for the acceptable number of *Legionella* in cooling tower water is hampered by these difficulties. The objective of this study was to investigate which protocol would result in the highest recovery of *Legionella* from cooling tower water with the lowest investment of manpower and culture media.
Methods: We compared several culture methods including the method described by the ISO and a method used by a Dutch reference laboratory with the procedure as prescribed by the Dutch standardisation institute (NEN). Thirty cooling towers were sampled in the summer in both cities.
Results: Seventy percent of the cooling towers were contaminated with *Legionella* species in numbers ranging from 200 to >106 CFU/L. Failure to isolate *Legionella* was mostly caused by overgrowth of the inoculated media by other non- *Legionella* bacteria.
Conclusion: Methods using filtration, heat decontamination and the use of highly selective culture media with glycin containing antibiotic supplements resulted in the highest recovery rates of *Legionella* from these water samples. The method as described by the ISO was among the best methods and required relatively little materials and labour. Our results confirm that setting a standard for this type of water may be difficult.

P1262 Clinical study of a new chromogenic medium for the isolation and the direct identification of *Staphylococcus aureus*

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Objective: The current using of chromogenic medium in laboratories, allows visualising and rapidly identifying various microbial species, especially in polymicrobial samples. The aim of the study was to evaluate the biological performances of a new chromogenic medium *S. aureus* ID bioMerieux (SA ID) for easy identification of *S. aureus* colonies by a green colouration.
Methods: The study was carried out to test this new medium in parallel with the CHROMagar *S. aureus* medium (CM SA) on which *S. aureus* colonies are pink. Columbia blood agar was used as reference method. In total, 515 clinical samples were inoculated directly on the three media either with a swab or with a 10 μL loop (100 blood cultures, 108 nasal swabs, 102 suppurations (swabs or liquid pus), 73 ear, nose, throat and bronchial samples, 29 urines, 54 faeces, 49 genital samples). The three media were incubated for 24 h in aerobic conditions at 35°C. The colonies were identified by the catalase test, the Slidex staph plus kit and the coagulase test. In case of discordant results, additional identification tests were performed.
Results: 129 *S. aureus* were isolated: 112 *S. aureus* were recovered on both chromogenic media, there were two false negatives on SA ID, and 15 on CM SA. There were 18 false positives on SAID (nine of them are catalase negative and easily differentiated from *S. aureus* with regard to the thin aspect of the colonies). There were four false positives on CM SA. For SAID medium the sensitivity was 98.4%, the positive predictive value (PPV) was 87.6% (93% by eliminating the nine Gram-positive cocci obviously not *S. aureus*), the specificity was 95.3%, and the negative predictive value (NPV) was 99.5%. For CM SA medium, the sensitivity was 88.4%, the VPP was 96.6%, the specificity was 98.9%, and the VPN was 96.2%.
Conclusion: The results clearly indicate that chromogenic media enable a simple and rapid method of recovering specific bacterial species even in polymicrobial samples or when the pathogenic bacterial species are present in small quantities among abundant commensal flora. The sensitivity of the SA ID medium is greater than that obtained with CM SA, but on the other hand, because of this good growth capacity, the specificity and PPV for CM SA are slightly better. In addition SA ID inhibits most non-staphylococci Gram-positive bacteria as well as most Gram-negative bacteria with, only a few multi-resistant Gram-negative bacilli recovered.

P1263 Detection of *Helicobacter pylori*: evaluation of staining methods

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Objectives: To compare the sensitivity of traditional detection methods (haematoxylin and eosin (HE) stain, modified Giemsa stain) and immunohistochemistry using a commercially available anti- *Helicobacter pylori* antibody (Dako, Denmark) in diagnosis of *H. pylori* infection in gastric biopsy and resection specimens.
Methods: Thirty gastric antral biopsies showing chronic gastritis together with tissue blocks from gastrectomy specimens for duodenal ulcer were reviewed histologically. The paraffin sections were stained with HE and modified Giemsa and immunoenzymatically treated by alcaline phosphatase anti-alkaline phosphatase (APAAP) method for the identification of *H. pylori*.
Results: The presence of chronic gastritis was confirmed in the 30 gastric biopsy specimens. A diagnosis of duodenal ulcer was confirmed in the mucosa from the gastrectomy specimens. The HE, modified Giemsa and immunoenzymatic treated sections were carefully examined for the presence of *H. pylori*. HE-stained *H. pylori* appeared as slightly basophilic, spiral-shaped organisms attached to the apical surface of the surface mucous cells. However, curved bacteria were only detected when found in great numbers. Using a modified Giemsa stain, the spiral-shaped bacteria of *H. pylori* stained blue, were attached to the brush border of the gastric foveolar epithelial cells and inside gastric pits. In some cases masked bacteria hidden within mucous were obvious only in immunostained preparations (red deposits). Similarly, in modified Giemsa treated sections, coccoid forms, which were particularly seen in sections from resection specimens, caused some uncertainty. These coccoid *H. pylori* were obvious in immunostained preparations. *H. pylori* was identified in 34.4% sections stained with HE, but it could be identified with greater frequency in sections stained with modified Giemsa (71.8%). It could be detected at a still greater frequency in sections stained with APAAP (90.6%). In all cases the bacteria were more prominent and easier to detect in the immunostained sections than in sections stained tinctorially.
Conclusion: Immunoenzymatic staining of tissue sections by the APAAP procedure is a highly sensitive and specific method. At the same time, it is a reliable and easy to perform tool for detecting this organism in gastric biopsy and resection specimens.

P1264 Antibiotic-associated colitis: value of colonic ultrasound in diagnosis and follow-up

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Background: Thickening of bowel wall at Ultrasound (US) is an aspecific sign in infectious-inflammatory bowel diseases. We evaluated US of colon as a tool for diagnosis and follow-up of antibiotic associated colitis (AAC).
Patients and methods: We retrospectively studied clinical and imaging records of 32 patients (18 males; age: 27–80 years) with the diagnosis of AAC. All diagnosis were based on patients'history of massive antibiotic intake preceding clinical signs (diarrhoea, abdominal pain, fever). Causes of antibiotic intake were: portosystemic encephalopathy in 13 cirrhotis patients, recent surgery in eight patients, respiratory tract infections in nine, liver abscess in two. Cultures of blood and faeces were performed in all patients. In 10 cases, stools were tested for toxin of *Chlostridium difficile* by enzyme-linked immunosorbent assay (ELISA). All patients underwent colonic US within 24 h from admission at our institution and every 3 days during the following 15 days. Thirteen patients also underwent colonoscopy. All patients were treated with Metronidazole (125 mg $\times$ 4/day) and Vancomycin (500 mg $\times$ 4/day) for 10 days.
Results: ELISA test for *Chlostridium difficile* toxins was positive in 4/10 (40%) patients. All blood cultures were sterile. In 4/13

patients (31%) colonoscopy showed mucosal pseudomembranes, pathognomonic pattern for the diagnosis of Pseudomembranous colitis while in nine patients it showed an aspecific pattern of acute colitis. US showed normal (≤4 mm) colonic wall thickness in seven patients and colonic wall thickening (ranging from 8 to 33 mm) in 25/32 patients (78%). Nineteen of 32 (59%) patients had colonic wall thickness <15 mm while in 13/25 (41%) patients it was >15 mm.
Follow-up: Remission of symptoms occurred in all patients within 2–11 days (mean: 3.6 days). Persistence of symptoms was significantly longer in patients with colonic wall thickness >15 mm (mean: 5.1 days) than in patients with <15 mm (mean 2.5 days) at US. Four cirrhotic patients relapsed 7–14 days after completion of therapy. Three of them (75%) still showed persistence of colonic wall thickening after the 10 days of metronidazole-vancomycin therapy. Normalisation of colonic wall thickness (≤4 mm) at US occurred in 29 patients within 3–9 days. None of these patients relapsed after completion of treatment.
Conclusions: Although aspecific, thickening of colonic wall at US seems to be a very sensitive tool for diagnosis and follow-up of AAC.

P1265 Can the use of a microbiological screen of dental implants predict implant failure?

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Objectives: To determine whether measuring the bacterial load of dental implants or detecting the presence of selected microorganisms can predict implant failure.
Methods: The presence of selected 'target' organisms (*S. aureus*, beta-heamolytic streptococci, coliforms, anaerobes, and *Candida* species) was detected from paper point samples taken from 19 patients in areas around failing (FI) and healthy implants (HI). Each paper point was placed into 1 ml of sterile nutrient broth and quantitatively cultured using the following media: blood agar, heated blood agar (enumeration), aerobic Mannitol salt agar, aerobic MacConkey agar, anaerobic neomycin agar, Fastidious anaerobe agar with a 5 ug metronidazole disc and Sabourauds agar. Anaerobic bacteria were identified to the level of Gram morphology and pigmentation; all other isolates were identified to species or genus level.
Results: Nine patients had >10 CFU/mL of one target organism present in either implant sample; five patients had >10 CFU/mL of more than one target organisms present and five patients were found to have no target organisms present. Of the 14 patients who had one or more than one target organisms present, the association with implants was as follows, 14% patients had the target organism present in the HI only, 43% had the target organisms present in the FI only and 43% had the target organisms present in both implants. Implant bacterial loads were as follows: 53% patients had a higher bacterial load around the FI; 11% had a higher bacterial load around the HI; 11% had the same bacterial load in both implants and in 26% details of the bacterial load were not recorded.
Conclusion: Measuring the bacterial load of dental implants may be a more predictive measure of implant failure than screening for the presence of specific microorganisms.

P1266 Acute primary diagnosis of malaria on medical wards in a non-endemic country using the Binax Pf/Pv (R) rapid antigen detection test

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Microscopy for malarial parasites may be unreliable in non-endemic countries. We assessed the Binax Pf/Pv (R) rapid antigen test for routine clinical use where training and routine of alternating staff and other factors may affect the promising outcome in controlled studies. The test was consecutively performed on patients with suspected malaria admitted to nine Danish hospitals both at bedside and in the laboratory. Results were compared with expert microscopy of Giemsa-stained smears. Samples from 83 patients were examined by at least one rapid test. All 11 *Plasmodium falciparum* and two of three *P. vivax* infections were detected. There were three false negative (one *P. malariae*, one *P. vivax*, and one *P. ovale*), but no false positive tests. Some found it difficult to perform the test. In particular, the presence of three bands, which may be interpreted as either *P. falciparum* or a mixture of *P. falciparum* with another species, was confusing. In addition, quantification and staging of the parasites was not possible. Rapid testing for malaria may be used for preliminary diagnosis of *P. falciparum*, but microscopy should always be performed as soon as possible, irrespective of the antigen test result. The study continues and updated results will be presented.

P1267 Differentiation of *Candida albicans* from other yeast species using a new, simple and non-hazardous biochemical test kit

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Objectives: Owing to differences in antifungal susceptibility, it is important to rapidly distinguish *Candida albicans* from other yeast species in order to initiate effective therapy. The germ-tube test (GT) remains an important physiological test for the differentiation of *C. albicans* from other *Candida* spp. by its ability to produce pseudohyphae in serum at 37°C. However, this method requires incubation for 2–4 h followed by microscopic examination. Oxoid Biochemical Identification System (OBIS) albicans is intended as a simple and rapid replacement for GT, incubation time being reduced to 1 h without the need for subjective microscopic examination. It is a non-carcinogenic, two-stage biochemical test that does not use fluorogenic substrates, thereby, eliminating the need for UV detection, providing both safety and ease of use.
Methods: The test kit detects two enzymes, beta-D-galactosaminidase and L-proline aminopeptidase using chromogenic substrates. Both these enzymes are produced by *C. albicans* whereas one or both enzymes are absent in other yeast species. OBIS albicans was tested with 310 clinical yeast isolates. One hundred and seventy-two were tested using OBIS albicans and GT and 138 were tested using OBIS albicans and a chromogenic detection medium (CHROMagar Candida) (CHROM).
Results: In the first part of the study, for the differentiation of *C. albicans* ($n = 132$), OBIS albicans had a sensitivity and specificity of 100 and 95%, respectively, and GT 100 and 92.5%. In the second part of the study, for the differentiation of *C. albicans* ($n = 76$), OBIS albicans had a sensitivity and specificity of 100% and CHROM 100% and 98.7%.
Conclusion: OBIS albicans is recommended as a less subjective, more rapid and user-friendly alternative to the germ-tube test, being easier to use than other commercially available rapid biochemical tests for the presumptive identification of *C. albicans* from pure culture clinical isolates.

P1268 Identification of chlamydial antigens by means of the phage surface display technique

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Chlamydophila pneumoniae is an emerging pathogen with high serological prevalence. To date, serological tests use whole elementary bodies as antigen, which are less standardised and subjective with regard to interpretation. The use of pure recombinant and thus well-characterised antigens could contribute to the improvement of the diagnostic specificity and sensitivity, which

are not satisfactory at the moment. A random phage surface displayed genomic library of *C. pneumoniae* TW 183 with a complexity of 7.2×10^5 independent clones was generated and affinity selected for IgG-specific clones, using two different highly seropositve serum pools. Pool 1 was composed of eight sera from atherosclerosis patients and pool 2 of 20 sera from healthy seropositive donors. Restriction analysis and sequencing of affinity enriched clones revealed that the sequence for the polymorphic membrane protein family A (pmp 19) was present in ~70% of the clones enriched using both serum pools. Open reading frames encoding porphobilinogen deaminase and serin/threonin protein kinase were present in 11 and 5% of the enriched clones, respectively. Seven other proteins were found at lower frequency. No clones were enriched when sera from *C. pneumoniae*-negative donors were used for selection. Reverse transcription-PCR analysis showed that all enriched sequences are indeed transcribed during infection of HEp-2 cells. The three predominant proteins (pmp 19, porphobilinogen deaminase, serin/threonin protein kinase) might represent promising antigen candidates for the development of general diagnostic reagents for the detection of *C. pneumoniae* infections.

P1269 Detection of *Chlamydophila pneumoniae* in acute exacerbation of COPD patients by real-time PCR and MIF test

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Objective: *Chlamydophila pneumoniae* is a common respiratory tract pathogen known to be associated with pneumonia, bronchitis, pharyngitis and asthma. We aimed to study the role of *C. pneumoniae* infections in patients with acute exacerbation of chronic obstructive pulmonary disease (AECOPD) using Micro-Immunoflourescence (MIF) test and Real-Time polymerase chain reaction (PCR) method.
Methods: Real-Time PCR was used to determine *C. pneumoniae* from throat swab ($n = 69$) and sputum ($n = 61$) samples from 69 patients with AECOPD and throat swab ($n = 64$) samples from 64 healthy individuals. *C. pneumoniae* antibodies was performed by MIF test in both groups to determine acute infection.
Results: *C. pneumoniae* infection was detected in 15 (21.7%) patients by MIF test and/or real-time PCR. Twelve (17.4%) patients of AECOPD group were positive for *C. pneumoniae* by real-time PCR. Serological evidences of acute infection were found in five (7.2%) patients by MIF test. Two patients were positive for *C. pneumoniae* infection with both PCR and MIF methods. On the other hand, acute infection was not detected in control group by MIF test. Five (7.8%) persons of control group were positive for *C. pneumoniae* by PCR. The presence of acute *C. pneumoniae* infection in between two groups was statistically significant ($P < 0.05$). However, no statistically significant differences were found between sputum and throat swab samples by using PCR ($P > 0.05$). *C. pneumoniae* IgG seropositivity was 82.6% in AECOPD patients and 62.5% in control group ($P < 0.05$). Chronic persistent *C. pneumoniae* infection was detected in 43.5% of AECOPD patients and 23.4% of control ($P < 0.05$).
Conclusion: In conclusion, our findings suggested that *C. pneumoniae* might have a role in the pathogenesis of AECOPD. In addition, real-time PCR may be a useful method in the diagnosis of *C. pneumoniae* infection and could be utilised for both of sputum and throat swab samples.

P1270 Isolation and characterisation of oral *Actinomyces* species from patients with periodontal diseases

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The present study was carried out on 100 patients with periodontal diseases referred to the Faculty of Dentistry, Tehran University of Medical Sciences. The main purpose of the study was the isolation and characterisation of the oral *Actinomyces* species, which are residents in dental plaque. The samples were selected based on the following criteria: periodontal plaque with deep pocket (>3 mm), no antibiotic therapy for a period of at least 2 weeks, and lack of systemic diseases. In this study 100 specimens were collected during a period of 6 months and the following results were obtained. One species of *Actinomyces viscosus* and two species of *Actinomyces naeslundii* were isolated from the patients with gingivitis and periodontitis. Of the 100 patients with gingivitis and periodontitis, aged between 18 and 57 years, 46 were males (46%) and 54 were females (54%). The peak incidence of the diseases (35%) was in the third age grouping (31–40) and the low incidence (10%) was in the first age grouping (<20). Forty patients (40%) complained of gum disease and bleeding with incidence of (42.5%) in female and (57.5%) in male. The results of this study suggest that: *Actinomyces* species may contribute to the aetiology of periodontal diseases and further work is required.

P1271 Evaluation of a new Oxoid ELISA for the detection of enteric adenoviruses

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Objectives: The Oxoid Adenovirus ELISA is a new rapid method designed to detect adenoviruses in faecal samples using specific antibodies raised towards the hexon protein. Sensitivity, specificity, positive and negative predictive values (PPV and NPV) of the assay were determined using faecal samples submitted for routine investigation of adenovirus infection.
Methods: One hundred and four archived faecal samples that were analysed had previously been confirmed as adenovirus positive by electron microscopy (EM). Fifty-four adenovirus negative faecal samples were also analysed. These either naturally contained or were spiked with non-adenoviral intestinal viruses, bacteria, fungi and protozoa. Faecal samples were thoroughly mixed with dilution buffer in a sample tube. A total of 50 μl of the sample and conjugate were added to each well of the microtitre plate and the plate incubated at room temperature for 30 min without shaking. The plate was washed five times with wash buffer before adding the substrate reagent and developing at room temperature for 15 min. Stop reagent was then added and the plate read at 450 nm with the plate reader blanked on air. Each assay run was validated by ensuring that positive and negative (diluent) control absorbances were greater than 1.000 and less than 0.150, respectively. Individual sample results were determined using the following equation: Cut-off = Diluent Control absorbance + 0.1. Samples were determined as positive if absorbance values were at least 10% greater than the cut-off value, negative if at least 10% less than the cut-off value and equivocal if within 10% of the cut-off value.
Results: Sensitivity and specificity of the Oxoid Adenovirus ELISA were 94.2 and 98.1%, respectively, in comparison to EM. Six samples that had previously been confirmed as positive by EM produced negative results with the Oxoid assay. On re-examination by EM adenovirus particles were absent from these samples. PPV and NPV for this assay were calculated to be 99.0 and 89.8%, respectively.
Conclusion: The Oxoid Adenovirus ELISA is a simple, rapid method for the diagnosis of adenovirus infection in stool samples. Incubation steps are at room temperature and do not require shaking. Results are available within 3 h with a permanent copy of results for laboratory records. The assay will be particularly useful for laboratories that process large numbers of samples but lack access to EM.

P1272 Evaluation of a new Oxoid ELISA for the detection of rotavirus

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Objectives: The Oxoid Rotavirus ELISA is new rapid method designed to detect rotavirus in faecal samples using specific anti-

bodies raised towards the major capsid protein (VP6). The sensitivity, specificity, positive and negative predictive values (PPV and NPV) of the assay were determined using faecal samples which had been submitted for routine investigation of rotaviral infection.
Methods: One hundred and one archived faecal samples that had previously been confirmed as rotavirus positive by routine laboratory analysis were analysed. Fifty-eight rotavirus negative faecal samples were also analysed. These either naturally contained or were spiked with non-rotaviral intestinal viruses, bacteria, fungi and protozoa. Faecal samples were thoroughly mixed with dilution buffer in a sample tube. A total of 50 μl of the sample and conjugate were added to each well of the microtitre plate and the plate incubated at room temperature for 30 min without shaking. The plate was washed five times with wash buffer before adding the substrate reagent and developing at room temperature for 15 min. Stop reagent was then added and the plate read at 450 nm with the plate reader blanked on air. Each assay run was validated by ensuring that positive and negative (diluent) control absorbances were greater than 1.000 and less than 0.150, respectively. Individual sample results were determined using the following equation: Cut-off = Diluent control absorbance + 0.2. Samples were determined as positive if absorbance values were at least 10% greater than the cut-off value, negative if at least 10% less than the cut-off value and equivocal if within 10% of the cut-off value.
Results: The Oxoid Rotavirus ELISA demonstrated good sensitivity (97.1%) and specificity (96.3%). Three samples (2.8%) positive for rotavirus on receipt in the laboratory produced negative results with the Oxoid ELISA. On examination by electron microscopy, rotavirus particles were absent from these samples. PPV and NPV for this kit were calculated to be 95.3 and 94.5%, respectively.
Conclusion: The Oxoid Rotavirus ELISA is a simple, rapid method for the diagnosis of rotavirus infection in stool samples. Incubation steps are at room temperature and do not require shaking. The assay is easy to perform enabling results to be obtained within 3 h with a permanent record of the results.

Mycobacterial disease: pathogenesis and therapy

P1273 Reaction to cheese during tuberculosis treatment

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We present a case of tyramine syndrome or 'cheese reaction' after ingestion of parmesan cheese by a patient assuming isoniazid for breast tuberculosis (TB). A 26-year-old nurse had a microbiological diagnosis of breast tuberculosis (TB) and was started on standard four-drug antituberculosis therapy. Seven weeks later she developed episodes of facial flushing, respiratory distress ('constriction on throat'), headache and asthenia. The episodes were noticed while dining and lasted 30 min. Other than the symptoms associated to facial flushing, the patient reported mood alteration since the beginning of TB treatment, alternating periods of euphoria and depression, insomnia and somnolence. The patient was informed about the possibility that her symptoms were associated with cheese and black wine intake in association with isoniazid use. She confirmed the regular consumption of different kinds of cheese, especially parmesan cheese. The patient was encouraged to avoid the consumption of cheese and TB therapy was maintained. The patient by her own initiative decided to test the association of her symptoms with the ingestion of cheese. Few days later she ate a large amount of parmesan cheese: after 10–15 min she presented the typical reaction, that in this occasion lasted 1 h. Since that episode, the patient avoided eating ripened cheese and remained free of symptoms. She successfully completed her TB treatment course with no further adverse events. The most frequent symptoms/signs associated with tyramine syndrome are skin flushing, palpitation and elevated blood pressure, usually beginning some minutes after ingestion of food rich in tyramine. Tyramine syndrome is a reaction secondary to the inhibitory effect of isoniazid on monamino oxidase (MAO) activity, causing high concentration of tyramine at the nervous system level. In the case presented here the control of symptoms was achieved by restricting cheese from the patient's diet. Physicians should be aware of the syndrome to avoid unnecessary interruption of TB treatment.

P1274 The predictive value of serum procalcitonin levels in patients with pulmonary tuberculosis

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Objectives: The aim of our prospective study was to evaluate the predictive value of serum procalcitonin (PCT) levels in diagnosis of pulmonary tuberculosis (PTB).
Methods: We measured serum PCT levels on admission and 6 months after antituberculous therapy (ATT) in 75 male adult patients aged 19–80 years (mean age 23.1 years), who had bacteriologically diagnosed (smear and culture positivity) active PTB (study group). Also, 75 male adult healthy individuals aged 18–56 years (mean age 23.3 years) with no physiological complaints were enrolled (control group).
Results: The measured serum PCT levels were within normal range both in healthy individuals (mean 0.15, range 0.02–0.47 ng/mL) and in patients after 6 months of ATT (mean 0.15, range 0.03–0.43 ng/mL). Serum PCT levels had been slightly high on admission in patients with PTB, before the implementation of ATT (mean 0.47, range 0.02–1.09 ng/mL) ($P < 0.05$) in comparison with controls and patients who had ATT.
Conclusion: We thought that the serum PCT level on admission was not a reliable indicator in the diagnosis of PTB even if statistically meaningful results are obtained. Serum PCT assay may be helpful to differentiate PTB from bacterial community acquired pneumonia. Elevated admission serum PCT levels (>1 ng/mL) could help clinicians to limit the number of tuberculosis cultures to be processed and to decrease the number of empiric ATT.

P1275 Determination of IL-4, IL-6, IL-8 interleukins and TNF-α in patients with tuberculosis during treatment

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Aim: The aim of the study was the determination of interleukins IL-4, IL-6, IL-8 and TNF-α in patients with tuberculosis.
Patients and methods: Determinations were performed in sera of 12 patients (five women, seven men, age 15–87) successively as follows: first, before the beginning of treatment; second 5–7 days after beginning; third 10–15 days after beginning; fourth 60 days after beginning and fifth 180 days after beginning of treatment. All determinations were performed by ELISA.
Results: The results were as follows. For IL-4, mean values were 20.9 (min 3, max 45), 40.3 (min 12, max 73), 67.5 (min 28, max 146), 25 (min 2, max 58), 2.7 (min 0, max 6) successively. For IL-6, mean values were 83.3 (min 22, max 180), 103 (min 34, max 200), 209 (min 135, max 347), 132 (min 56, max 190), 4.2 (min 2, max 7) successively. For IL-8, mean values were 39.7 (min 8, max 88), 45.8 (min 2, max 77), 47.2 (min 12, max 90), 44 (min 14, max 76), 42 (min 18, max 88) successively. For TNF-α, mean values were 27.9 (min 2, max 125), 41.9 (min 3, max 154), 100 (min 23, max

242), 45 (min 5, max 140), 10 (min 0, max 134) successively. Results were statistically evaluated with SPSS system and Friedmann's test was used. For IL-4, chi square was 40.5 (Asymp. Sig: 0.001); for IL-6, chi square was 42.3 (Asymp. Sig: 0.00); for IL-8, chi square was 3.3 (Asymp. Sig: 0.5), for TNF-α chi square was 29 (Asymp. Sig: 0.006).
Conclusions: Results show that there are significant differences between successive measurements of IL-4, IL-6 and TNF-α in sera of patients with tuberculosis during treatment, while differences in successive measurements of IL-8 are not statistically significant. Values were significantly raised in the third measurement of all markers with the exception of IL-8.

P1276 Intracellular growth of clinical isolates of *M. tuberculosis* with different ability of dissemination

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Objectives: There is scarce information about the virulence mechanisms used by *M. tuberculosis* to evade host inmune defences. Restriction fragment length polymorphism (RFLP) typing is a useful tool for differentiating isolates and identifying specific strains as responsible for tuberculosis outbreaks. Assessment of the virulence of these isolates by measuring their ability to grow in human macrophages may be useful to explain the extent of spread of a particular strain in the community.
Methods: We selected four clinical isolates from patients with pulmonary tuberculosis based on RFLP typing; isolate 1072 was the most prevalent strain cultured in our laboratory and it has been responsible for several outbreaks; strain 4590 was isolated in a small cluster, and strains 7506 and 3625 are unique strains that cause disease in only one patient. We have used virulent *M. tuberculosis* strain H37Rv as control.
Method: Macrophages were prepared from peripheral blood mononuclear cells (PBMC) from healthy donors, and 5×105 adherent cells/well were plated in 24-well plates in RPMI with 10% fetal bovine serum and 10% of human serum and cultured for 7 days to mature into macrophages. Cells were infected with suspensions of the different strains of *M. tuberculosis* studied using a ratio of 1 bacillus/20 cells. At time points 1, 3, 7 and 20 days, infected macrophages were lysed and the intracellular mycobacterial growth was analysed by a quantitative real-time PCR assay that amplified a region of the 85 kDa antigen.
Results: All the clinical strains tested presented an intracellular growth higher than the control strain H37Rv; after 20 days of culture strain 1072, responsible for several clusters, and the unique strain 7506, grew significantly ($P < 0.001$) more than strain H37Rv.
Conclusions: The ability of *M. tuberculosis* strains to grow in human macrophages is an individual characteristic of each strain and our data indicated that it is not always correlated with the dissemination between population.

P1277 Sigma factors and hspX expression could be markers of stress adaptation and reactivation of growth of *Mycobacterium tuberculosis* in acidified cultures

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Objectives: *M. tuberculosis* is the paradigm of intracellular bacteria and the explanation of its behaviour inside the phagosome may be crucial to deeply know its pathogenesis. Too many efforts have been done to elucidate these mechanisms, but few studies have been performed related to the acidification of phagosome. An *in vitro* model was made to resemble these acidic conditions inside macrophages. We studied the survival of *M. tuberculosis* and the genetic expression within a range of pHs from 4.5 to 6.5 for 15 days.
Methods: *M. tuberculosis* strain H37Rv was grown in 7H9 medium to mid-log phase and frozen in aliquots. Following, one frozen aliquot was grown in non-acidified 7H9 medium until exponential and steady-state phase. Starting from the same culture, inoculation in previous acidified medium (to final pHs of 4.5–5–5.5–6 and 6.5 at 25°C) was performed by triplicate at the point of exponential and steady-state phase. We determined the CFUs from acidified cultures and the mRNA expression by real-time PCR of 64 genes related to heat shock proteins, metabolism and sigma factors for 15 days.
Results: Related to CFUs from the exponential phase derived cultures of pH 4.5–5 and 5.5, no survival was detected (<10 UFCs) after 7 days. Noteworthy, a progressive drop of hspX, acr, rpoA and recA was obtained until day 7 that was undetectable. Interestingly, sigE, sigF, sigG and sigH expressions increased in cultures of pH 4.5 from day 7. Survival of the steady-state phase derived cultures of pH 4.5–5 was very low (<100 UFCs) after 7 days too, but curiously CFUs from pH 5.5 cultures were maintained constant from the beginning and started to grow from day 7. Again, CFUs of pH 6 and 6.5 were able to grow normally, reaching superior numbers than the same pH cultures from exponential phase. The same profile of sigma factors was obtained as in exponential phase. On the contrary, hspX showed superior levels from day 7 in pH 5.5.
Conclusion: A well-defined profile of sigma factors could provide *M. tuberculosis* an ability to survive in low pHs conditions. Increase of hspX expression might be a marker of the adaptation of bacilli to stressing conditions and growth reactivation. In regard to survival, we also confirm that cultures submitted to stress conditions, like the steady-state derived cultures, are able to best adapt their metabolism to acidification.
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P1278 The effects of azathioprine and prednisolone on cytokine expression in the skin and blood of severe leprosy type 1 reaction patients

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Objectives: To compare the cytokine production in skin lesions and blood of severe leprosy type 1 reaction (T1R) Nepali patients taking azathioprine and or prednisolone. To relate these findings to the clinical state of the patients and the drug treatment administered.
Method: Forty patients were randomly assigned to a 12-week treatment with either AP (12 weeks azathioprine at 3 mg/kg/day plus 8-week reducing course prednisolone starting at 40 mg/day) or P (12-week reducing course prednisolone starting at 40 mg/day). Levels of leprosy antigen-induced tumour necrosis factor (TNF-α), interleukin-10 (IL-10) and gamma-interferon (IFN-γ) in whole blood was assessed by ELISA before, during and after treatment. The effects of treatment on the cellularity and cytokine (TNF-α, IL-10, IL-2) expression in the skin lesions of patients were studied using immunohistochemistry. Two skin biopsies were taken from each patient to cover the period before, during and after treatment.
Results: No difference was found between the two treatment groups with respect to cytokine expression in the skin or blood. For both groups, median levels of whole blood TNF-α and IFN-γ fell during treatment, but as the dose of prednisolone decreased, the level of cytokine expression increased. Neither treatment had any significant effect on median whole blood IL-10 or skin TNF-α expression. In both groups, median levels of IL-10 and IL-2 in the skin decreased during and after treatment. No correlation was found between cytokine expression in the skin and blood and clinical outcome.
Conclusion: We conclude that the combination of azathioprine and short-course prednisolone produces no difference in cytokine expression in the skin and blood of T1R patients compared with a longer course of prednisolone. This study agrees with previous

work that prednisolone is associated with a dose-dependent reduction in TNF-α and IFN-γ in the blood, but this does not correlate to TNF-α expression in skin lesions of T1R patients. Our data also shows a reduction in both Th1 and Th2 cytokine expression in skin lesions with treatment. This indicates that improvement in T1Rs may not only be due to the down regulation of Th1 cytokines and the up regulation of Th2 cytokines. We found no correlation with level of pro-inflammatory and anti-inflammatory cytokines and clinical outcome in T1R patients giving no indication of possible immunological markers for those patients with recurrent or relapsing T1Rs.

P1279 Serum total adenosine deaminase level in active pulmonary tuberculosis in comparison to other infectious diseases in Iran

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Objective: In order to have an acceptable rapid test helping the clinicians in the diagnosis of active pulmonary tuberculosis, we evaluated the importance of elevated serum adenosine deaminase in active pulmonary tuberculosis vs. other infectious diseases.
Methods: We measured serum total adenosine deaminase level in three groups: (1) cases of active pulmonary tuberculosis who were confirmed by positive sputum smears for acid-fast bacilli in association with compatible clinical and radiological findings, (2) cases of other infectious diseases including Brucellosis, Endocarditis, Salmonellosis, meningitis confirmed by clinical findings and related laboratory tests and (3) healthy controls. Serum adenosine deaminase levels were measured before treatment was started. Data analysis was performed by chi-square; ANOVA and LSD. The significance level was evaluated for P value of less than 0.05.
Results: We evaluated 51 (21 females and 30 males aged 47.7 ± 19 years) cases of active pulmonary tuberculosis, 11 (six females and five males aged 44.7 ± 21 years) cases of other infectious diseases and 50 (14 females and 36 males aged 48.4 ± 11 years) cases of healthy individuals. Mean serum total adenosine deaminase level in pulmonary tuberculosis (42.4 ± 21.5 IU/mL) and other infectious diseases (38.3 ± 23.4 IU/mL) was meaningfully more than controls (26.6 ± 8.2 IU/mL), ($P < 0.0001$ and $P < 0.03$ respectively), but the difference in pulmonary tuberculosis and other infectious diseases was not statistically significant. There was no significant difference in age and gender between the above groups.
Conclusion: We conclude that serum total adenosine deaminase increases in infectious diseases but it cannot differentiate pulmonary tuberculosis from other infectious diseases.

P1280 Paradoxical response in infliximab-treated patients with disseminated tuberculosis

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Objective: Infliximab is a tumour necrosis factor antagonist that has been approved for the treatment of certain autoimmune diseases. A number of opportunistic infections have been reported in patients treated with this drug. The most frequent is tuberculosis, usually extrapulmonary and disseminated. The aim of our study is to describe the clinical features of the disease and response to antituberculous therapy in patients with disseminated tuberculosis who have been treated with infliximab.
Design: Retrospective cohort study.
Methods: Between 1999 and 2002, we reviewed all patients that had been treated with infliximab and had developed active tuberculosis infection in three acute referral centres from different geographic locations, in Spain.
Results: In the cohort of 284 patients that had been exposed to this drug, six patients (2.1%) developed disseminated tuberculosis, and four (67%) presented a paradoxical response while on antituberculous treatment. Two were women and mean age was 43.5 (SD13.3) years. Indications for infliximab therapy included rheumatoid arthritis (2), ankylosing spondylitis (1) and Crohn's disease (1). The most frequent clinical presentation was swelling of pre-existing adenopathic mass. The median number of months with infliximab treatment before tuberculosis diagnose was two (range1–24). The mean time interval between initiation of antituberculous treatment and the development of paradoxical reaction, was 9 weeks. Two cases were treated with anti-inflammatory agents (steroids and NSAIDS) with progressive improvement. In the other two cases local excisional surgery was required.
Conclusions: Our experience suggests that patients with TB after infliximab exposure have a high probability of having a paradoxical reaction and that this may be due to immunologic mechanisms. Reinitiation of infliximab once active TB is controlled should be contemplated. Physicians should be aware of this increased risk and when paradoxical reaction is suspected, consider the use of corticosteroids.

P1281 Long-term efficacy of a 6-month treatment regimen (6HR2Z) for HIV-negative tuberculous meningitis

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Objective: To investigate the long-term efficacy of a 6-month treatment regimen (6HR2Z) for tuberculous meningitis in the HIV-negative population.
Methods: All HIV-negative patients with tuberculous meningitis attended in our Centre entered a 6-month treatment protocol (6HR2Z) and were prospectively followed in our Tuberculous Unit for a period of 70.1 ± 55.5 months (only one case was lost to follow-up 3 months after entering the study). The diagnosis of tuberculous meningitis was based on cerebrospinal fluid (CSF) examination, cultures and/or pathology of specimens. Demographics as well as a complete set of tests (including BCG, Mantoux test, chest X-ray, and brain CT scan) were collected in each case. Daily doses employed in the 6HR2Z protocol were: isoniazid (H), 5 mg/kg; rifampin (R), 10 mg/kg; and pyrazinamide (Z), 30–35 mg/kg.
Results: We recruited 19 patients with HIV-negative tuberculous meningitis. There were seven females and 12 males (mean age, 40 ± 22.5 years; range, 11–74 years). All patients had CSF findings compatible with tuberculous meningitis. Four patients (21%) had also pulmonary tuberculosis. In 10 cases (53%), the final diagnosis was established by the demonstration of *M. tuberculosis* in CSF culture; in two additional cases, *M. tuberculosis* was recovered from culture of expectorated sputum specimens (not from CSF). Distribution of clinical stage was as follows: stage I (eight cases; 42%), stage II (eight cases; 42%), and stage III (three cases; 16%). All patients were initially treated according to the 6-month 6HR2Z protocol. In addition, nine patients received adjunctive therapy with corticosteroids. As to the outcome, 14/18 (78%) patients were cured, two (11%) died of tuberculosis during the first days of treatment, and two (11%) died of unrelated causes 2 and 41 months after starting treatment, respectively. Three patients developed complications during the treatment, two developed brain tuberculomas (treatment was prolonged for 3 more months in one case), and one developed a tuberculous brain abscess, which was removed surgically (treatment was prolonged for 6 more months). Thirteen of the 19 patients who entered the study (68%) completed the treatment as initially planned (i.e. 6-month therapy); only four of them had some late sequelae (anosmia, one case; paraparesis, one case; headache, two cases).
Conclusion: Our results suggest that a 6-month treatment regimen (6HR2Z) is optimal for tuberculous meningitis in the HIV-negative population.

P1282 ***Mycobacterium tuberculosis* complex sigma factor H gene expression during pulmonary tuberculosis treatment**

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Objectives: The objective of our study was to evaluate the specific transcriptional activity of mycobacteria contained in the sputum expectorated by patients with pulmonary tuberculosis during their treatment.
Methods: This was done by measuring the mRNA level for *Mycobacterium tuberculosis* extracytoplasmic sigma factor H with the aid of the real-time Q-RT-PCR performed on ABI PRISMTM 7700. The study group consisted of 52 patients (mean age 44 ± 11, $M = 39$, $F = 13$) with pulmonary tuberculosis confirmed by AFB (+) and culture (+). The sputum samples were taken for the analysis before anti-tuberculous treatment and after 4 weeks of treatment (standard set: RFP+INH+PZA+SM).
Results: In the beginning of the therapy the proportion between sigma H (sigH) mRNA positive and negative sample was equal. After 1 month of poly-therapy 70% of samples were sigH transcript positive and culture was still positive in 94%. The mean copy number of sigH transcript per 1 μg of total RNA was twice higher during treatment than at the beginning of the therapy (4342 (1659) vs. 8034 (2007), respectively; mean (SEM)).
Conclusion: The increasing level of sigH factor transcript during treatment could reflect the probable role of this co-factor of transcription in stating defence reactions of Mycobacteria to anti-mycobacterial action of immune system and specific drug therapy. Discovering the mechanisms of interaction between this pathogen and the host in the context of introduced therapy could help in better management of tuberculosis and finding new way of treatment.

Mycobacterial disease: multiresistant tuberculosis

P1283 **Diagnosis of multiresistant tuberculosis by detection of mutations in rpoB and inhA by real-time PCR**

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Objective: The emergence of multidrug-resistant tuberculosis (MDR), characterised by the resistance to at least rifampin (RIF) and isoniazid (INH), made the application of molecular methods to early detection of resistant *M. tuberculosis* strains a priority. More than 96% of RIF-resistant strains have specific mutations within an 81-bp region of the *rpoB* gene, encoding for the â subunit of the RNA polymerase. In contrast, molecular characterisation of INH resistance is complex, and has been associated with mutations in at least four different genes: *katG*, *inhA*, *kasA* and *ahpC*. The aim of this study was to detect in a single tube RIF and INH resistance-associated mutations in *rpoB* and *inhA* genes by real-time PCR using Light-Cycler (Roche).
Methods: *rpoB*, *inhA* and *katG* and genes of 30 MDR strains were sequenced on an ABI-Pris (Applied Byosistems). We found that most of our isolates had no mutations in *katG* but mutations occurred frequently in *inhA* gene. Primers and hybridisation probes were designed for these more frequently found mutations in *rpoB* and *inhA* genes and 30 resistant strains were analysed, comparing to control sensitive strains, in a Light Cycler.
Results: Melting curves, by presenting changes in T_m, allowed the detection of the mutation in the *rpoB* gene in 100% of the strains, being 90% at the codon 531 and 10% at the codon 516. Among the *inhA* gene we found the searched mutation in 63.3% of the strains.
Conclusions: The method provided a rapid and accurate way to detect RIF-resistance among *M. tuberculosis* strains, and to predict the presence of MDR, most RIF-resistant strains are MDR. As to INH resistance, we found that in this study *inhA* gene is the predominant target of mutation.

P1284 **Can phenotypic susceptibility testing for *M. tuberculosis* be performed before identification?**

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Objectives: Given the increase in multidrug-resistant *M. tuberculosis* (MDRTB) throughout the world, new tests seem necessary to promptly detect resistant strains. Thus, we prospectively evaluated a modified MGIT-SIRE(R) method (Mycobacterial growth indicator tube-streptomycin, isoniazid, rifampin, ethambutol) based on DIRECT decontaminated specimen susceptibility to isoniazid (INH) and rifampin (RIF) in patients infected with *M. tuberculosis* (MTB). We compared the results obtained, concerning time gain and accuracy, to the reference MGIT-SIRE method performed once the MTB was isolated and identified (here, as INDIRECT susceptibility test).
Methods: Starting from January 2003, we selected all strongly Acid Fast Bacilli (AFB)-positive smears (stronger than 50 AFB/field). Samples were decontaminated and cultured according to standardised procedures and DIRECT specimen susceptibility testing to INH and RIF was performed in MGIT using the protocol approved by the FDA for MTB susceptibility testing. Briefly, 500 μL of specimen was DIRECTLY inoculated into MGIT, using 0.1 μg/mL INH and 1.0 μg/mL RIF as critical concentrations. We used a 1×10^{-2} sample dilution as growth control. MTB isolates were identified by AccuProbe(R) (GEN-PROBE(R)) and their susceptibility tests (INDIRECT susceptibility tests) were performed using the MGIT-SIRE procedure (BD(TM)).
Results: We processed 26 samples in parallel with both the direct and indirect procedure. Compared results were as follows: 23 of 26 samples (88.5%) were susceptible to INH, whereas 25 (96.1%) were susceptible to RIF. The results of both procedures agreed 100%, giving our new DIRECT method an accuracy of 100%. The mean time to detection was 10.8 days using the DIRECT procedure, and 19.0 with the INDIRECT one.
Conclusions: The DIRECT specimen susceptibility test MGIT-SIRE is a new, rapid and accurate method to measure susceptibility to INH and RIF. This procedure provides results earlier than INDIRECT MGIT-SIRE, with a mean time gain of 8.2 ($P < 0.0001$). The DIRECT susceptibility test enables us to rule out MDRTB. These results could be very useful for the prompt recognition of MDRTB, especially in an area of high prevalence for resistant MTB.

P1285 **Antimicrobial susceptibilities of *Mycobacterium tuberculosis* strains isolated from clinical specimens in Crete, Greece**

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Objective: To evaluate the activities of first-line antituberculosis drugs on 100 strains of *Mycobacterium tuberculosis* isolated from clinical specimens.
Methods: One hundred *Mycobacterium tuberculosis* strains were tested, which were isolated from different clinical specimens between 2001 and 2003. For the drug sensitivity study, the standard method of Canneti-Grosset was performed. The drugs tested were: isoniazid (INH), rifampicin (RF), ethambutol (ETB), streptomycin (SM), *para*-4-aminosalicylic acid (PAS), and pyrazinamid (PYZ).
Results: Resistance to one drug was observed in 14% of the isolates. SM, INH, ETH, and PYZ resistance rates were 6, 3, 2 and

3%, respectively. Resistance to two or more drugs (MDRTb) was observed in 5% of the isolates. One percent of the isolates was resistant to SM + IZH, 1% to SM + PYZ, 1% to SM + IZH + RF, 1% to SM + ETH + RF and 1% to SM + IZH + PAS.
Conclusion: Continuous surveillance on the susceptibility pattern to the antituberculosis drugs on local level is necessary for determining therapeutic regimens, important precondition for avoiding the appearance of MDR strains.

P1286 Malnutrition in multidrug resistant tuberculosis patients in the Philippines

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Objectives: Little is known about the nutritional status of patients with multidrug resistant tuberculosis (MDR TB), and whether nutritional status impacts treatment outcomes. Therefore, the purpose of our study was to describe baseline nutritional status of patients with MDR TB in the Philippines, and to determine whether baseline nutritional status was associated with time to sputum smear and culture conversions.
Methods: Records of MDR TB patients currently receiving treatment at the Makati Medical Center DOTS-Plus Pilot Project in Makati City, the Philippines were reviewed. Data collected included age, gender, occupation, weight, height, body mass index (BMI), drug regimen, and monthly sputum acid-fast bacillus (AFB) smear and culture results for the first 6 months of treatment. Time to smear or culture conversion was defined as the month at which the first of two negative smears or cultures was observed. Data were summarised using means and standard deviations or numbers and percentages. Log rank tests were used to compare time to sputum smear and culture conversion among those who were and those who were not malnourished. All data were analysed using the JMP statistical software package (SAS Institute, 2003).
Results: Among 43 patients, there were 23 (53.5%) males and 20 (46.5%) females; the mean age was 39.3 ± 13.6 years. At baseline, MDR TB patients had a mean BMI of 19.2 ± 4. Half of these patients were malnourished (BMI < 18.5), and 14 (33.3%) had moderate–severe malnutrition (BMI < 17). Four (9.3%) patients had positive smears after 6 months of treatment. Malnutrition was significantly associated with a longer time to sputum smear conversion ($P = 0.02$). Malnutrition was not associated with time to culture conversion ($P = 0.98$).
Conclusion: Malnutrition is common in Filipino MDR TB patients. Prevalence of malnutrition in these patients far exceeds national prevalence rates of 13.2% and 4.4% for malnutrition and moderate–severe malnutrition, respectively, in adult Filipinos. There was a significant association between malnutrition and slower sputum AFB smear conversion; correlation with culture conversion was not demonstrated, possibly due to the small number of patients studied. Further data on this population are currently being collected to better understand the relationship between nutritional status and MDR TB treatment outcomes.

P1287 Resistance problem in *Mycobacterium tuberculosis*: evaluation of the resistance of 166 *M. tuberculosis* strains against four major drugs

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Objectives: In our study, we aimed to detect the presence of *M. tuberculosis* with BACTEC MGIT (Mycobacteria Growth Indicator Tubes) (Becton-Dickenson) 960 system and reference method Löwenstein–Jensen (L–J) in clinical samples having suspect of pulmonary and extrapulmonary tuberculosis and also to determine the resistance of the isolated *M. tuberculosis* strains against four major antimicrobial drugs and to evaluate the primary and secondary resistance of the cases. The study period was between 2001 and 2003.
Methods: BACTEC MGIT 960 system uses a fluorescent compound that is sensitive to oxygen. The recovery of mycobacteria is faster in this method from the solid media (L–J). Antimicrobial susceptibility tests were performed with BACTEC MGIT 960 SIRE kit and L–J. We followed the test procedure in the MGIT kit and we used proportion method in L–J to detect susceptibility to streptomycin, isoniazid, rifampin and ethambutole.
Results: One hundred and sixty-six strains were identified as *M. tuberculosis* with conventional biochemical tests (niacin and catalase production). In 166 cases consisting of 141 newly and 25 previously diagnosed patients; drug resistance were seen in 23 (13.8%), which were 10 (7.09%) as primary and 13 (52%) secondary resistance. In one drug resistance, both primary and secondary resistances were detected, two drug resistance was seen only in primary resistance (one case), four drug resistance was seen only in secondary resistance (four cases) and three drug resistance was seen only in secondary resistance (one case). The highest cumulative drug resistance was found against streptomycin in primary and against rifampicin in secondary resistance.
Conclusions: Our results emphasises that high ratio of secondary resistance in public may be related to insufficiency of therapy and this may cause a serious increase of primary tuberculosis with new contaminations.

P1288 Recovery rate and drug resistance of mycobacterial strains by the BACTEC 460TB system during 2001–2003 in Kocaeli, Turkey

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Objectives: To investigate the rate of isolation and *in vitro* activity of antituberculous agents against *M. tuberculosis* isolated from human clinical samples.
Methods: From 2001 to 2003, 2576 samples have been processed for tuberculosis in Kocaeli University Hospital. All samples were cultured on Löwenstein–Jensen medium and in Middlebrook 7H12 broth medium after decontamination and concentration steps. Drug susceptibility tests to isoniazid (INH), streptomycin (S), ethambutol (E), and rifampin (R) were performed by using the radiometric BACTEC 460TB system.
Results: Out of the 2576 samples, 1690 were from the respiratory tract (sputum, pulmonary biopsy, bronchoalveolar lavage, bronchoalveolar brush specimen), 528 were urine, 283 were from body fluids (153 pleural, 51 peritoneal, 45 intraarticular, 34 CSF), 73 were from skin and tissue biopsies, two were gastric aspirate, and faeces. *M. tuberculosis* were isolated in 81 (3.1%) cases including redundant samples: 45% were from the respiratory tract, 25% were urine, 18%were body fluids, 12% were tissue biopsies. Six of the samples were MOTT identified from respiratory tract specimens (3), urine (2) and knee effusion material (1). Our results of susceptibility testing are summarised in Table 1.

Table 1. Susceptibility testing results of *M. tuberculosis*, during 2001–2003, in Kocaeli University, Turkey

Total testing number	91
Susceptible to all four drugs	64(79%)
Any streptomycin (S) resistance	–
Any isoniasid (INH) rersistance	8(9.8%)
Any rifampin (R) resistance	2(2.4%)
Any ethambutol (E) resistance	7(8.6%)
S monoresistance	–
INH monoresistance	6(7.4%)
R monoresistance	–
E monoresistance	6(6.1%)
Total monodrug resistance	11(13.5%)
Total INH + R resistance(MDR)	–
Total INH + E resistance	2(2.1%)
Total R+E resistance	1(1.2%)
Total polyresistance other than MDR	3(3.7%)

Conclusions: These data showed that, among first-line drugs, drug resistance is not yet a problem in therapy of tuberculosis for Kocaeli. INH resistance can be a big problem in future, while streptomycin is still active against all the isolates.

P1289 Drug resistance rates of *M. tuberculosis* strains from civilian and prison patients in Samara region, Russia

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Background: The true extent of drug resistant tuberculosis (TB) globally is unknown. We have initiated a study to determine the extent of resistance and the molecular epidemiology of drug resistance in Samara, one of 89 regions within Russia.
Design: Cross-sectional study of prison and civil TB patients in Samara. Phenotypic Rifampicin and Isoniazid resistance was determined in Samara and at MRU, London using the resistance ratio and absolute concentration methods. All cultures were analysed by spoligotyping.
Objectives: To determine true rates of primary and acquired drug resistance to first-line TB drugs and rates of multiple-drug resistance (MDR); to determine the epidemiology of *M. tuberculosis* strains in civilian and penitentiary sectors in Samara.
Results: A total of 3408 individuals with TB were enrolled. Isolates from 600 patients attending all TB institutions in Samara Region including 295 prisoners were tested. The prevalence of drug resistance (all chronic and new patients) across the Region was 61.2% (367/600), 50.3% (302/600), 46.9% (282/601), 26.8% (161/600) and 8.9% (23/260) to Inh, Rif, S, E and Z, respectively. The prevalence of MDR in all TB cases was 47.2% (283/600). Primary resistance among civilians was 33.7% (32/95), 22.9% (22/96), 28.1% (27/96), 11.5% (11/96) and 6.1% (3/49) to the above mentioned first-line drugs, respectively, and 20.0% (19/95) MDR. The primary resistance analysis demonstrated statistically significant difference between prisoners and civilians. Inh resistance was by 29.0% higher in the prisoners (95%CI 14–44), Rif resistance was by 15.0% higher (95%CI 1–29), S resistance by 43.3% (95%CI 27–60), E resistance by 21.9% (95%CI 6–37) and MDR by 17.9% (95%CI 4–32). However, no significant difference was identified between rates of acquired resistance among civilian and prison patients. Molecular epidemiological analysis demonstrated the dominance of Beijing strains – 65.4% (619/946). The prevalence of mono-resistance and MDR was significantly higher among Beijing isolates compared to non-Beijing strains: the difference was 33.4% (95%CI 25–41), 34.3% (95%CI 26–42), 26.9% (95%CI 19–35), 19.3% (95%CI 12–26) and 25.8% (95%CI 28–44), respectively, to Inh, Rif, S, E and MDR. The incidence of primary resistance is significantly higher among prisoners than civilian patients. The Beijing strain family predominates and is associated with higher rates of drug resistance.

P1290 Multicentre study on *Mycobacterium tuberculosis* susceptibility testing using BacT/ALERT automatic system

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Introduction: In our environment, tuberculose endemia is reducing by 12% annually but the effect of tuberculosis still remains high, above all, due to the immigrant population coming from Latin American, Asian and Eastern European countries, with the possibility of a greater rate of resistance to anti-tuberculosis drugs.
Objectives: The aim of this study is to verify the reproducibility of the antibiogram results in the system BacT/ALERT through a cooperative study undertaken by four laboratories belonging to the following Centres: H. La Paz (Madrid), H. Miguel Servet (Zaragoza), H. Rio Hortega (Valladolid) and H. Carlos III (Madrid).
Methods: The Central laboratory sent 20 strains with different degrees of sensibility to four indicated laboratories. These strains have been tested previously by the conventional method for the proportions in solid medium. The method used for the antibiogram was according to the manufacturer's recommendations. The final drug concentrations used were: 0.1 mcg/mL for isoniazid, 1 mcg/mL for streptomycin and Rifampicin and 5 mcg/mL for ethambutol.
Results: The following results were obtained from the 80 antibiograms performed by the four laboratories. (1) Complete agreement: out of the 80 antibiograms carried out, 78 were concordant (97.50%) and two were discordant (2.5%). (2) Agreement was 100% for Isoniazid and Rifampicin. (3) For Streptomycin and Ethambutol, agreement reached 95% (one false result resistant to Ethambutol and one false result resistant to Streptomycin). (4) The results were obtained within an average of 9 days.
Conclusions: According to the results obtained, the sensibility test carried out with the BacT/Alert 3D system is as accurate as the conventional procedure using the proportions in solid medium.

P1291 Detection of mutations at embB codons 306 and 497 and iniA codon 501 by PCR-RFLP for rapidly determining resistance to ethambutol in ethambutol-resistant clinical *Mycobacterium tuberculosis* isolates

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Objectives: Mutations at embB gene codons 306 and 497 and iniA gene codon 501 occur frequently in ethambutol-resistant *Mycobacterium tuberculosis* isolates. The aim of this study was to improve or develop PCR–restriction fragment length polymorphism (RFLP) methods for rapid screening of ethambutol-resistant clinical *M. tuberculosis* isolates carrying substitutions at these codon positions.
Methods: The *M. tuberculosis* H37Rv was used as the susceptible strain while well-characterised clinical isolates of *M. tuberculosis* with specific substitutions at embB codons 306 and 497 and iniA codon 501 were used as reference strains. The presence of mutations was detected by PCR amplification of the DNA region around the respective codon position followed by digestion with appropriate restriction enzymes to generate RFLPs.
Results: The PCR–RFLP performed with Nla III with the susceptible strain carrying ATG and the mutant strains with GTG, ATT and CTG at embB codon 306 yielded DNA fragments of expected sizes. The restriction digestion performed with Hae III differentiated the strains with mutation at the first codon position (GTG and CTG) and those with mutations at the third codon position (ATT). The PCR–RFLP performed with AlwN I and Hpy99 I with the susceptible and mutant strains for embB codon 497 and iniA codon 501, respectively, also yielded expected DNA fragment patterns. In a preliminary application to clinical isolates, the established methods correctly identified mutations at embB codons 306 and 497 and iniA codon 501 in ethambutol-resistant *M. tuberculosis* strains and the results were confirmed by direct DNA sequencing.
Conclusions: We have developed PCR–RFLP based methods for rapidly determining the substitutions at embB codons 306 and 497 and iniA codon 501. Since substitutions at these codon positions occur frequently in ethambutol-resistant clinical *M. tuberculosis* isolates, application of simple PCR–RFLP-based methods will result in rapid identification of resistant strains carrying these mutations.
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P1292 Molecular characterisation of isoniazid-resistant *Mycobacterium tuberculosis* strains from the Free State and Northern Cape provinces, South Africa

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Objectives: In South Africa the true extent of isoniazid (INH) and multidrug resistant tuberculosis (MDRTB) is unknown. A national

survey has reported an overall INH resistance of 6.3 and 1.6% MDR for new cases in the Free State (FS) province. However, INH resistance rates of 14.5–20% have been reported in localised, high incidence areas. MDR transmission and gene mutation data are limited. The aim of this study was to determine the strain diversity of and gene mutations in INH resistant strains isolated from the FS and Northern Cape (NC) provinces.
Methods: Genotyping was performed on 29 strains using DNA fingerprinting of the IS6110 insertion sequences and spoligotyping was done on seven (<5 bands) strains. Cycle sequencing of the *katG* and *rpoB* genes was performed on nine strains.
Results: DNA fingerprinting profiles showed seven strains with five or less insertions. From these, two strains in a cluster were confirmed by spoligotyping while another cluster was proved to consist of different strains. The remaining 22 strains contained nine to 18 copies of the IS6110 insertion sequences. Fingerprinting patterns were very diverse with 20 different profiles in 22 strains. One cluster (two patients) was evident in each province. At a 65% similarity index, patterns were still too diverse to suggest recent transmission. *KatG* mutation analysis of nine strains revealed three strains with a missense mutation at codon 315 changing AGC to ACC and two mutations were found at codon 463 (CGG to CTG; CGG to CCG). Two of the strains harbouring the 315 mutation were in the same cluster and investigations suggested interfamily transmission. *RpoB* gene analysis performed on 7/9 MDR strains revealed three strains with a missense mutation at codons 531, 526 and 516. Two strains had an addition at codon 514 and one at codon 522. These additions still have to be confirmed. One strain had no changes within the 81-bp region regarded as the core region for *rpoB* gene mutations. All strains were in different clusters.
Conclusion: The presence of no large clonal groups agrees with published data suggesting non-compliance as the major cause of resistance development. The cluster caused by interfamily transmission indicates an urgent need for active case finding in close contacts of infected patients. This study suggests that the DOTS program in the Free State and Northern Cape is successful, but continued surveillance of strain transmission and drug resistance in the general community remains imperative.

P1293 *rpoB* mutations in rifampin-resistant clinical *Mycobacterium tuberculosis* isolates from Turkey

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Objectives: Drug-resistant tuberculosis is a serious problem throughout the world. Resistance to Rifampicin (RIF) is mainly caused by the mutations in the hot-spot region of the *rpoB* gene coding the beta subunit of RNA polymerase. In this study, we aimed to detect the distribution of *rpoB* gene mutations in 81 RIF-resistant clinical *Mycobacterium tuberculosis* (MTB) isolates from TURKEY.
Methods: The hot-spot region of the *rpoB* gene of MTB was amplified by PCR using the primers BC35 (5′-ATCAACATCCG-GCCGGTGGT-3′) and BC41R (5′-TACACCGACAGCGAGCCGAT-3′) as described previously. Mutations leading to RIF-resistance were determined by automated sequence analysis.
Results: Seventy-three isolates (90.1%) were found to carry mutations in the amplified region, while eight isolates (9.9%) carried no mutations. Overall, 25 different missense mutations affecting 15 codons, and two deletion mutants were identified. Ten new mutations – six in the hot-spot region and four outside this region – were found. The codon numbers of the most frequently encountered mutations were 531 (45.7%), 526 (16%), 516 (12.3%) and 513 (11.1%). As a result, nearly 90% of the RIF-resistant MTB isolates were found to carry a mutation in the *rpoB* gene, Ser531Leu being the most frequent one.
Conclusions: Although molecular methods identify mutations leading to RIF-resistance very quickly; for the patients carrying no mutations in this region, results of the antimycobacterial susceptibility tests must be taken into consideration.

P1294 Use of PCR–SSCP for rapid detection of rifampin and isoniazid resistance-associated mutations in *Mycobacterium tuberculosis*, isolated in Suez Canal region, Egypt

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Objectives: Recently emerged multidrug-resistant MTB (MDR-TB) is a health problem that created a national concern. Most Egyptian hospitals do not perform susceptibility testing for MTB isolates. Empirical treatment with antituberculosis drugs prolongs the period of illness and infectivity due to emerging MDR-TB during therapy. PCR–single strand conformational polymorphism (PCR–SSCP) is evaluated in this work, as a rapid and non-expensive method for detection of MDR-TB.
Methods: Forty MTB strains were isolated from 75 patients with pulmonary TB (new and retreated cases) attending four main hospitals in the Suez Canal region of Egypt. MTB strains were identified by growth on LJ medium, biochemical activities, and amplification of IS6110 and IS1245 in a multiplex PCR. They were tested for susceptibility to rifampin (Rif), isoniazid (Inh), streptomycin (Sm) and ethambutol (Etb) by the standard agar proportion (AP) method. Isolates were assayed for Rif and Inh mutation-associated resistance by two separate PCR–SSCP assays. Mutations were detected in the 81-bp region of rpoB gene (for Rif) and 321-bp sequence of katG gene for Inh resistance.
Results: Rates of resistance to each drug by AP method were: 47.5, 45, 37.5, and 25%, for Sm, Inh, Rif, and Etb, respectively. Combined resistance to Rif and Inh (MDR-TB) was 35%, while resistance to four drugs was 17.5%. MDR-TB strains were isolated 74% of retreated cases and none from new cases. Resistance rates to each drug were higher in retreated cases. These correlations were statistically significant ($P < 0.05$). PCR–SSCP was successful in detecting rpoB gene mutants in 12 out of 15 Rif-resistant isolates; with sensitivity, specificity and overall predictivity of 80, 100, and 92.5%, respectively. It could also detect katG mutations in 13/18 Inh-resistant strains, with sensitivity, specificity and overall predictivity of 72.2, 100, and 87.5%.
Conclusions: PCR–SSCP might not be the most reliable assay to detect Rif-resistant MTB; but definitely it is not recommended for detection of Inh-resistant MTB. Combination with other molecular method – such as DNA sequencing – will enhance the sensitivity and predictivity of PCR–SSCP.

P1295 Tuberculosis caused by multiresistant strains of *Mycobacterium tuberculosis* in the Czech Republic. Microbiological, epidemiological and DNA analysis

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Objectives: Multidrug-resistant tuberculosis (MDR-TB), defined as a disease caused by *Mycobacterium tuberculosis* strains resistant to more antituberculous drugs (at least to isoniazid and rifampicin), gains in importance in the Czech Republic (CR). These TB cases refractive to causal therapy are associated with increased risk of spreading of the infectious agent among population.
Methods: The National Reference Laboratory for Mycobacteria collected, in total, 2813 *M. tuberculosis* strains isolated in Czech mycobacteriological laboratories in the period from 1999 to 2001. All strains were tested for susceptibility to first-line antituberculous drugs and the multidrug-resistant (MDR) group was also tested for susceptibility to second-line antituberculous drugs. The MDR strains were further analysed by DNA fingerprinting (Restriction Fragment Length Polymorphism – RFLP).
Results and Conclusion: In total, there were 39 MDR-TB cases. A total of 56 *M. tuberculosis* strains were isolated from these patients during the investigated period. On average, MDR-TB accounted for 1.96% of all TB cases in the CR. The most frequent type of the MDR-*M. tuberculosis* isolates was resistant to four first-line drugs (isoniazid, rifampicin, streptomycin, ethambutol) and was found

in 48.2% of all MDR-*M. tuberculosis* strains. Isepamicin, clofazimin, capreomycin and amikacin are considered to be the most promising second-line drugs according to the shown data. Based on RFLP profiles, 61.5% of strains were assigned to eight clusters while the other strains remained unclustered. There were no significant differences between the excretors of clustered and unclustered strains concerning geographical distribution and population structure. Comparison of RFLP profiles with the international database suggests uniqueness of Czech MDR-*M. tuberculosis* strains, showing the profiles not found elsewhere to date.

Miscellaneous issues

P1296 Chlorination of bacterial surfaces by *N*-chlorotaurine

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Objectives: *N*-chlorotaurine (NCT), an endogenous active chlorine compound, has broad-spectrum activity against microorganisms and seems to be of advantage in topical therapy of infections of e.g. the eye, the ear, and the skin. Although at least 20–30 min of incubation time are necessary for killing of bacteria by NCT, their virulence is already attenuated within 1 min as demonstrated in an *in vivo* model. Rapid chlorination of bacterial surface ('chlorine cover') has been assumed for explanation and been investigated in this study.
Methods: Washed bacteria were treated for 1–3 min with NCT. Subsequently the oxidant was removed by centrifugation or filtration, and the chlorination measured spectrophotometrically after addition of 5,5'-dithiobis (2-nitrobenzoic acid).
Results: NCT produced a chlorine cover of about 3×10^{-16} mol Cl+/CFU on *S. aureus*. This cover did not kill the microorganisms. It was influenced by pH and coating time as well as by the kind of test strain. Chlorine covers were surprisingly stable, e.g. for hours at 0°C in saline, even without a remarkable reduction of viability. Regarding the consequences, chlorine-covered *S. aureus* was phagocytosed by human granulocytes at a slightly higher rate than a mock treated one. Moreover, chlorine covered *E. coli* lost viability after transfer into active human serum in contrast to mock treated ones.
Conclusion: Chlorination of bacterial surfaces by the mild oxidant NCT may explain the rapid loss of virulence before killing takes place. Chlorine-covered microorganisms seem to be more susceptible to the human defence system, which may be important for both the clinical application of NCT and its role in innate immunity as a product of leukocytes.

P1297 Amelioration of the prokinetic effect of erythromycin on oesophageal motility by atropine

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Background: Erythromycin and its analogues are a group of antibiotics, which may be indicated in the clinical use. Side effect of this group of drugs is mainly concentrated in the field of gastroenterology. They are known to have a prokinetic effect. This undesirable effect may limit the use of this drug.
Aim of work: The aim of this work was to determine the block effect of atropine on the receptors with the drug intake in order to ameliorate the undesirable side effect of the drug when we give erythromycin in combination with atropine.
Methods: This work was done on three groups of healthy volunteers, each group consisted of 10 coherent healthy volunteers. In group one, erythromycin was taken and oesophageal motility was assessed. In group two, atropine was taken with erythromycin. In group three, placebos were taken with erythromycin. Oesophageal motility was assessed by solid state catheter on a fasting state and the upper oesophageal sphincter (UES) function was assessed by pressure of UES and pharyngeal pressure and all the standard parameters. The body of the oesophagus pressure and the standard parameters were measured and the lower oesophageal (LES) pressure and relaxation were measured in all the groups. Statistical data were analysed according to standard student *t* test and significant difference was evaluated. The dose of erythromycin was 500 mg orally once daily for 5 days. Atropine dose was 15 mg/kg orally daily for the same period. The motility studies were performed at the end of the study.
Results: At 5% level of significance the data showed that significant changes were found after atropine intake. The increased motility test produced by erythromycin in group one was significantly reduced in group two ($P < 0.05$), while in group three, there was no significant changes than in group one ($P > 0.05$).
Conclusion: Erythromycin is one of the macrolide antibiotics that stimulate activity by binding at receptor on the intestinal muscle cell. This stimulating motility effect is considered in the evaluation of the side effect of this antibiotic. In this work this effect is showed to be ameliorated by the antagonistic action atropine on the receptor. From this work we can conclude that atropine should be given with erythromycin if you want to abolish the undesirable effect unless there is contraindication to atropine.

P1298 Impact of prolonged treatment with trimethoprim-sulfomethoxazole on the human gut flora

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Introduction: Trimethoprim-sulfamethoxazole (TMP-SMX) is a broad-spectrum antibiotic active against a wide variety of bacteria, *Pneumocystis carinii*, and some protozoa, but non-active against anaerobes. There are limited data on the effect of this drug on the gut flora after very prolonged treatment.
Objectives: To describe the impact of TMP/SMX on the gut flora of a patient who was mistakenly treated with TMP-SMX (800/160 mg bid) for 2 years.
Case report: A 43-year-old, mentally ill, man presented with a 3-day history of fever (39°C) and dysuria. The patient had urine incontinence, due to a traumatic lumbar spinal fracture, had an indwelling urinary catheter and was receiving TMP-SMX for urinary tract infection prophylaxis (one loading dose before and one after any catheter change). However, by mistake, he was continuously taking the drug daily for 2 years. Urinalysis showed increased protein with numerous leucocytes and bacteria. Urine culture yielded an extended-spectrum β-lactamase (ESBL) producing strain of *Escherichia coli*. Blood cultures were negative. Quantitative stool cultures for bacteria and yeasts yielded *Candida albicans* 2×10^5 CFU/g, *Bacillus licheniformis* 9×10^9 CFU/g, *Enterococcus faecalis* 1.1×10^{10} CFU/g, *Enterococcus faecium* 2×10^{11} CFU/g, *Bacteroides ovatus* 2.5×10^{10} CFU/g, *Bacteroides uniformis* $>10^{12}$ CFU/g, and *Clostridium acetobbytulicum* 1.2×10^{10} CFU/g.
Conclusions: This unique case confirms the preservation of the anaerobic gut flora during TMP-SMX treatment and suggests that this agent is a reasonable treatment option when gut colonisation resistance is required. The emergence of an ESBL producing strain of *E. coli* was apparently the result of the suppression of Gram-negative aerobic flora. *Enterococcus* spp. was not affected.

P1299 Ecological effects of pivmecillinam on the normal vaginal microflora

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Objectives: It has been shown that mecillinam affects the normal oropharyngeal, intestinal and skin microflora to a minor extent. The effect on the vaginal microflora is not known. The objective of this project was to study the ecological impact of pivmecillinam on the normal vaginal microflora.
Methods: Eighteen healthy women, 24–40 years old, with one sexual partner and not being infected with sexually transmitted pathogens were included in the study. The day of ovulation was determined during three subsequent menstrual cycles. Microbiological and clinical examinations were performed on the day of ovulation and on day 3 in all cycles and also on day 7 in cycles 1 and 2. The clinical examinations included observation of vaginal and cervical epithelium, photographing of cervix, pH- measurements and inspection of any discharge. Vaginal specimens were collected with sterile swabs at each visit. The specimens were diluted in pre-reduced medium, diluted 10-fold and inoculated on selective and non-selective agar plates. All different colony types were counted and identified to genus level. Pivmecillinam was administered 200 mg t.i.d. for 7 days starting on the day of ovulation in cycle 2.
Results: Anaerobic and facultative Gram-positive rods, mainly species of lactobacilli and actinomyces, dominated the vaginal microflora. Lactobacilli were only isolated sporadically in five of the women, all of whom were also colonised with *Gardnerella vaginalis*. *G. vaginalis* was further isolated from seven subjects. The aerobic microflora was dominated by species of corynebacteria. Seven women were colonised with *Escherichia coli* in low numbers in one to three samples. Four women were colonised in cycle one only, one in cycle one and on day 1 in cycle 2, and two women were colonised on day 3 and on day 7 in cycle 2, respectively. *Candida albicans* was isolated from four women and *C. dublinensis* from two. Five of these women had a microflora with a high percentage peroxidase-producing lactobacilli (>80%) while one subject had no H_2O_2-producing strains. Four of the women were colonised in all or in seven of eight samples, one was colonised on days 3 and 7 in cycle 2 and one women in cycle 3 only. There were variations in numbers of microorganisms between the menstrual cycles but not related to the administration of pivmecillinam.
Conclusion: Administration of pivmecillinam did not have any major ecological impact on the normal vaginal microflora.

P1300 Risk of adverse birth and neonatal outcomes for pregnant users of pivmecillinam – a population-based cohort study

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Objectives: Pivmecillinam is widely used by pregnant women in the Nordic countries for treatment of urinary tract infections. Long-term treatment with pivmecillinam can lead to carnitine deficiency. Carnitine is an essential cofactor in mitochondrial metabolism and carnitine deficiency may cause metabolic disturbances in the newborn. The existing epidemiological data on use of pivmecillinam during pregnancy are limited to a single cohort study of 414 pregnant women, based on data from our dataset. Unexpectedly, in this analysis, we found an increased risk of low Apgar score in the offspring of women who had redeemed a prescription for pivmecillinam during the third trimester, odds ratio 2.32 (95% confidence interval (95% CI) 0.30–18.16). All other risk estimates in the study were close to one. The relatively wide 95% CI for low Apgar score and the fact that sample size is a critical parameter in the study of drug-induced birth defects, made us perform a larger observational study in order to achieve greater statistical power and to add additional neonatal outcomes. We, therefore, extended the dataset used in the former study in order to examine the risk of adverse birth and neonatal outcomes among offspring of Danish women, who had redeemed prescriptions for pivmecillinam during pregnancy.
Methods: In a population-based cohort study based on data from the North Jutland Prescription Database, the Birth Registry, and North Jutland County's Hospital Discharge Registry, Denmark, we examined the risk of congenital malformations, preterm delivery, low birth weight, stillbirth, low Apgar score, hypoglycaemia and respiratory distress syndrome among pregnant users of pivmecillinam. The cohort included 2031 women who had taken up prescriptions for pivmecillinam during pregnancy, and 61 628 women with a livebirth or a stillbirth after the 28th week of gestation, who did not use pivmecillinam during pregnancy.
Results: The adjusted odds ratios for outcomes associated with use of pivmecillinam were: congenital malformations 0.83 (95% CI 0.53–1.32), preterm delivery 0.96 (95% CI 0.79–1.18), low birth weight 0.79 (95% CI 0.52–1.20), stillbirth 1.19 (95% CI 0.30–4.80), low Apgar score 1.17 (95% CI 0.37–3.66), hypoglycaemia 1.02 (95% CI 0.52–1.98) and respiratory distress syndrome 0.86 (95% CI 0.43–1.74).
Conclusion: In conclusion, use of pivmecillinam during pregnancy did not seem to increase the risk of adverse birth and neonatal outcomes.

P1301 Influence of ertapenem on cell activation and co-stimulatory molecule expressions

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Objectives: The influence of a novel parenteral carbapenem, ertapenem, on co-stimulatory molecule expression was examined *in vitro* and *in vivo*.
Methods: Spleen cells obtained from BALB/c mice 10 days after immunisation with 8.0 μg of haemocyanin absorbed to 4.0 mg of aluminium hydroxide were cultured in the presence of 100.0 μg/mL of haemocyanin and various concentrations of ertapenem. We examined the influence of ertapenem on cell activation by examining the proliferative response of cells and cytokine production. We also examined the influence of ertapenem on co-stimulatory molecule (CD40, CD80 and CD86) expressions on cultured splenic B-lymphocytes induced by *in vitro* antigenic stimulation using flow cytometry. Splenic B lymphocytes were obtained from these mice 24 h after antigenic challenge, and co-stimulatory molecule expressions were examined by flow cytometer.
Results: Cell activation induced by *in vitro* antigenic stimulation was suppressed by ertapenem when cells were cultured in the presence of more than 1.0 μg/mL of the agent. Addition of ertapenem at a concentration of 0.5 μg/ml into cell cultures also suppressed co-stimulatory molecule (CD40, CD80 and CD86) expressions on splenic B lymphocytes, which was enhanced by antigenic stimulation *in vitro*. Ertapenem administration for 4 weeks clearly suppressed the enhancement of CD40 and CD86 (but not CD80) expressions on splenic B lymphocytes induced by antigenic stimulation *in vivo*. This suppressive activity of ertapenem on co-stimulatory molecule (CD40 and CD86) expressions was further strengthened by the treatment of mice for 8 weeks. Long-term treatment with ertapenem also suppressed CD80 expressions, which was not suppressed by 4-week treatment.
Conclusion: The present results suggest that ertapenem exerts its immunomodulating effects through suppression of both cell activation and co-stimulatory molecule expressions induced by antigenic stimulation. These suppressive activities of ertapenem might contribute, in part, to the therapeutic mode of action of ertapenem on inflammatory diseases.

Paediatric infections

P1302 Risk factors for candidiasis in the neonatal unit: a matched case–control study

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Background and objectives: Certain aspects of modern neonatal intensive care, such as antibiotic administration or central catheter use, have been associated with *Candida* infection. There is a lack of information regarding the role of particular antimicrobial agents in the development of neonatal candidiasis, while the association of central catheter use and other risk factors with *Candida* infection has not been consistently confirmed in multivariate analysis models. The aim of this study was to improve our knowledge of risk factors associated with neonatal candidiasis, with emphasis on those related to medical care.
Methods: A 1:2 matched case–control study was performed. Cases were infants admitted to our neonatal unit (NICU) from 1998 to 2002 with *Candida* spp. isolated from blood, cerebrospinal fluid or suprapubic urine aspirate. Controls were matched to cases on birth weight, age on admission and date of admission to the NICU. Exposure to risk factors, including various antimicrobial agents, placement of different types of central venous or umbilical catheters, duration of parenteral nutrition and mechanical ventilation, length of stay in the unit (LOS) and history of necrotising enterocolitis, were analysed using the NICU database. We also assessed whether neonates colonised with antimicrobial (ceftazidime)-nonsusceptible Enterobacteriaceae (ANE), including extended spectrum beta-lactamase producing strains, are at increased risk of *Candida* infection. Information regarding colonisation with ANE was obtained from surveillance rectal cultures.
Results: Sixty infants with *Candida* infection were matched (among which 31 had *C. albicans* and 21 had *C. parapsilosis*). Multivariate analysis revealed mechanical ventilation and parenteral nutrition as independent risk factors for candidiasis. Ampicillin and vancomycin administration was associated with *C. albicans* infection. Use of central catheters, LOS, colonisation with ANE and history of necrotising enterocolitis were not independent risk factors for candidiasis in the multivariate model.
Conclusion: Parenteral nutrition and mechanical ventilation should be judiciously used in preterm infants. Changes in antibiotic policy in the NICU, including restriction of ampicillin and vancomycin use, may prove beneficial for the prevention of candidiasis and should be further investigated.

P1303 Secular trends in aetiology of neonatal sepsis (1991–2001)

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Objective: To investigate the spectrum of organisms causing neonatal sepsis in Bahrain and to assess the sensitivity to antibiotics.
Methods: The medical records of all infants who had positive blood culture from 1991 to 2001 in neonatal intensive care unit (NICU) in both Salmaniya Medical Complex (SMC) and Bahrain Defence Force Hospital (BDF), Bahrain have been reviewed.
Results: A total of 335 (4.1%) children had culture proven bacteraemia. The main agents isolated were coagulase negative *Staphylococcus* (CoNS) in 138 cases (40%) followed by *E. coli* in 35 cases (10%), *S. aureus* in 28 cases (8%) and Group B *Streptococcus* (GBS) in 26 cases (0.2% per thousand live birth), and *K. pneumoniae* 24 (7.2%). An increasing, high percentage (5.7%) of *Candida albicans* isolation was also noted as a cause for special concern. During 1999–2001 there was an increase in resistance to more than three antibiotics in CoNS. All GBS were sensitive to Penicillin G, Erythromycin and Clindamycin. *Klebsiella* and *Enterobacter* spp. showed resistance to many of the antibiotics tested, posing difficult therapeutic choices.
Conclusion: Our results differ appreciably from other studies in developed countries regarding GBS. The role played by GBS in our setting is modest. Therefore, universal prenatal screening to detect vaginal and rectal GBS colonisations is unwarranted. It can be considered in the presence of additional risk factors (premature delivery, premature rupture of fetal membrane or fever during delivery). Specifically tailored polices must be defined according to the local epidemiology. The threat of fungal infection must be carefully tackled.

P1304 Investigation of *Streptococcus pyogenes* strains isolated from children with fatal STSS

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The late 1980s *Streptococcus pyogenes* (GAS) was presented with severe infections associated with shock, bacteraemia, acute respiratory distress syndrome and death in a significant rate. The most serious infection, streptococcal toxic shock syndrome (STSS) has attracted special attention because of its lethality. The aim of our study was to investigate the M-serotypes as well as the sensitivity to antibiotics and the presence of pyrogenic exotoxin genes (spe genes) of STSS strains. GAS isolates from three children fulfilling the criteria for STSS were tested. The age and sex of the little patients were a girl 5, a boy 2.5 and a girl 3.5 years old. All three cases were fatal. The first girl was immunocompromised, having acute lymphoblastic leukaemia (ALL), the boy had asthmatic bronchitis treated by bronchodilators and the second girl was phenotypically healthy. Two of the isolates originated in blood and the remaining one in pharynx. One of the blood stains was also isolated from pleural fluid. The work out of these GAS strains included Lancefield serogrouping (TRANSLAB UK kit), serum opacity factor, T-serotyping (anti-T sera from SERVA Ltd,Prague), sensitivity to antibiotics (penicillin, vancomycin, chloramphenicol, rifampicin, erythromycin, clindamycin) by disk diffusion method, double disk test (Becton Dickinson's disks) and MIC (Etest,AB Biodisk). M-serotyping and spe genes were tested by conventional and molecular techniques (RSIL,HPA,London). STSS isolates belonged to M12, M28 and M84 serotypes. No strain was resistant to penicillin, vancomycin, chloramphenicol and rifampicin. Resistance to macrolides of iMLS phenotype attributed to the M84 strain isolated from the girl with ALL. The strains were speB and speC positive but speA negative. Serotypes M12 and M28 are among the 10 most frequent ones connected to STSS worldwide, but they are not as common as M1 and M3.M1 serotype was not found, as it was expected from the literature and its predominance in invasive GAS infections encountered in our hospital.

P1305 Beneficial effects of immediate treatment of group A beta-haemolytic streptococcal pharyngitis with antibiotics

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Objectives: A beneficial effect of antibiotic therapy on the clinical course of group A beta-haemolytic streptococcal (GABHS) pharyngitis has been demonstrated in the past several years. As GABHS pharyngitis is a frequently encountered problem, we thought the question of treatment impact on symptomatic response and on recurrent infection should be investigated.
Methods: Sixty children aged 1–12 years (24 girls, 36 boys) with culture positive for GABHS pharyngitis were enrolled in a randomised prospective study comparing the consequences of immediate

vs. delayed treatment (after 48–56 h) with penicillin. There were 30 patients (18 M, 12 F) in both immediate (I) and delayed (D) treatment groups. Patients who were previously treated with antibiotics and carriers were excluded. All patients were assessed for the existence of clinical symptoms (fever, dysphagia, loss of appetite) and clinical findings (cryptic/exudative tonsillitis and anterior cervical lymphadenopathy). ASO titres (>250 IU/mL) were assessed for streptococcal antibody response. All patients underwent therapy with penicillin (50 000 IU/kg/day). Symptoms were assessed after 2 days. Throat cultures were also repeated. Complications and recurrences were recorded after 1 and 3 months.
Results: In both groups fever was encountered in all the patients enrolled in the study. After 48–56 h, seven (23%) in I group and nine (30%) in D group had fever. Dysphagia was observed in 76% in I group and 9% of D group patients at the beginning. Clinical findings and improvement in these findings were similar in both groups. After 48–56 h, six (20%) in the I group and 24 (80%) in the D group were still culture-positive for GABHS. This result was significant ($P < 0.05$). Although it has been reported that penicillin treatment seemed to prevent development of type specific immunity to GABHS, ASO titres was not depressed in the I group after therapy. In the month following documented evaluation of GABHS, no recurrence or complication occurred in both groups. Similar results were obtained after 3 months.
Conclusions: We suggest that early initiation of antibiotics for GABHS will reduce acute symptoms and secondary spread. The risk of recurrence rate related to diminished streptococcal immunity in the early treated children was not observed in our group.

P1306 An evaluation of once daily cefdinir 25 mg/kg oral suspension in children with acute otitis media at risk of persistent or recurrent otitis media

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Objective: This phase 2/3, open-label, noncomparative, multi-centre study, assessed the efficacy of once daily CEF in the treatment of children with AOM at risk of PROM.
Methods: Children aged 6 months to 4 years with signs/symptoms of AOM at risk of PROM (characterised by ≥2 of the following: antibiotics in previous 3 months, age ≤2 years, day-care attendance or siblings/household contacts age ≤8 years), diagnosed by pneumatic otoscopy and tympanocentesis, received once daily CEF for 10 days. Children were evaluated pretreatment (Day 1), on therapy (Days 4–6), end of therapy (Days 12–14) and follow-up (Days 25–28). Repeat tympanocentesis on Days 4–6 was used to assess bacteriologic response.
Results: A total of 447 children were enrolled in the US, Israel and Latin America. Fifty-seven percent were male, 64% were ≤2 years of age. For the 227 children clinically and bacteriologically evaluable, 58% were male and 74% were ≤2 years of age; 13% had received pneumococcal conjugated vaccine. Forty-two percent of these children had ≥3 AOM infections within the past 12 months (including present infection) and 56% had received treatment for AOM within previous 3 months. Fifteen percent had multiple pathogens isolated pretreatment. Forty-seven percent of *S. pneumoniae* isolates were penicillin non-susceptible (PNSP). Bacteriologic eradication on Days 4-6 was achieved in 159/226 (70%) children and 188/262 (72%) of all pathogens were eradicated. Bacteriologic response in children with a single pathogen was 148/193 (77%). Eradication of penicillin susceptible, intermediate and resistant *S. pneumoniae* was 84% (46/55), 65% (17/26) and 39% (9/23), respectively; eradication of *H. influenzae* was 84/123 (68%). Overall clinical response for clinically and bacteriologically evaluable children on Days 12–14 was 82% and for *S. pneumoniae* and *H. influenzae* it was 75 and 82%, respectively. Clinical response on Days 25–28 was 142/167 (85%). Clinical response for children with pretreatment negative and positive cultures was 97 and 82%, respectively. CEF was discontinued due to an adverse event in only 3% of children. The most common adverse events were diarrhoea (13%) and vomiting (7%), most of mild severity.
Conclusions: This study specifically evaluated children with AOM at risk for PROM. In this high-risk population, CEF demonstrated good activity against penicillin susceptible *S. pneumoniae*, but decreased activity against PNSP, and a moderate effect against other pathogens. Further studies are needed to evaluate modified dosing or formulations of CEF in this high-risk population.

P1307 Epidemiology of *Streptococcus pneumoniae* causing acute otitis media in young children in the Czech Republic

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The increasing prevalence of multiple antibiotic resistances makes prevention by vaccination a logical approach. Capsular based pneumococcal vaccines are unsuitable for use in children under 2 years. Preliminary epidemiological data are required before studying the efficacy of a candidate pneumococcal multi-valent conjugate vaccines against acute otitis media (AOM) in the Czech Republic. Serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F are covered in various candidate vaccines.
Objective: To evaluate the prevalence of *Streptococcus pneumoniae* serotypes and their antibiotic resistance in <2 years old children suffering from AOM.
Method: Between October 1999 and November 2000, samples of middle ear fluid (MEF) were collected by tympanocenthesis from 310 children <2 years old, originating from 12 areas and diagnosed with AOM. MEF were analysed for bacterial identification in culture. Out of 143 AOM cases diagnosed with *S. pneumoniae*, 141 were typed. Capsular typing of pneumococcal strains was performed by quellung reaction using serotype-specific antisera. MIC method was used for resistance testing.
Results: *S. pneumoniae* was isolated from MEF specimens as causing pathogen of AOM in 46%. Serotypes 3, 19F, 14 and 23 represented 48% of pneumococcal otitis observed during the survey. All isolates were sensitive to amoxicillin/clavulanic acid. Resistant strains were found in 1% to penicillin, clindamycin and erythromycin, in 9% to chloramphenicol, 11% to doxycycline and trimethoprim/sulfamethoxazol.
Conclusion: In spite of very low resistance in *S. pneumoniae*, serotypes causing AOM in the Czech Republic pneumococcal conjugate vaccine is promising approach to control AOM. Eleven-valent vaccine may protect up to 75% of pneumococcal serotypes causing AOM, assuming an ideal efficacy, what represents 34% of all AOM diagnosed in children <2 years old.

P1308 Invasive serotypes of *Streptococcus pneumoniae* in nasopharynx of healthy children

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Objectives: Pneumococcal nasopharyngeal carriage is important because of its relation both to development of the disease and to spread of the pathogen. The most common nasopharyngeal carriage serotypes are the same as the invasive isolates, although the rank order of specific seroptypes may be different. Moreover, the serotype distribution of nasopharyngeal isolates is usually predictive of invasive isolates in a given population. We examined prevalence and antimicrobial resistance of invasive serotypes of *S. pneumoniae* in nasopharyngeal carriage among healthy children attending day-care centres (DCCs).
Methods: Throat and nose swabs obtained from 241 children of aged 3–5, attending four DCCs were plated onto selective sheep blood agar with 5 mg/L gentamicin. Pneumococci were identified by colony morphology, susceptibility to optochin, bile solubility and slide agglutination test (Slidex PneumoKit, BioMerieux), and

serotyped using antisera from Statens Serum Institute. Drug susceptibility of isolates was determined by disk diffusion method according to NCCLS or by the E-test (AB Biodisk).
Results: Nasopharyngeal carriage of *S. pneumoniae* was found in 51% of the children. The paediatric serogroups (6, 9, 14, 19, 23) constituted 77.2% of all 123 isolates. A total of 36.6% (45/123) of the *S. pneumoniae* strains were relatively resistant to penicillin (RRSP) and 8.1% (10/123) – highly resistant to penicillin (HRSP). All 45 RRSP strains belonged to serotypes 6B, 9V, 14 and 19F whilst nine HRSP had serotype 14 and 1 HRSP – 15B. The tested pneumococci were resistant to co-trimoxazole (52%), tetracycline (35%), erythromicin (25%), clindamicin (25%) and chloramphenicol (27.6%). Resistance to at least three antibiotic classes (MDR – multidrug resistance) was found in 42 isolates (34.1%) and all of them belonged to the paediatric serogroups. Among MDR strains, 61.9% were non-susceptible to penicillin.
Conclusion: Children in DCCs may constitute an important reservoir of resistant strains and may contribute to its spread in the community. It is important for communities to obtain the accurate and current knowledge of local antibiotic resistance patterns to determine appropriate empirical approach to pneumococcal infections in an era of rapidly increasing resistance of *S. pneumoniae* to currently available agents.

P1309 Resistance profile of *S. pneumoniae* and beta-lactamase production in *H. influenzae* and *M. catarrhalis* isolated from 258 children with upper respiratory tract infection in Southern Brazil – 2002

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Objective: Establish the susceptibility profile of *S. pneumoniae* and the beta-lactamase production of *H. influenzae* and *M. catarrhalis* from children with upper respiratory tract infection (URTI) in Brazil.
Methods: Samples (one per patient) were selected from patients less than 5 years old during the 2002 period. All subjects had clinical diagnosis of URTI and a positive culture result for at least one of the selected pathogens (*S. pneumoniae*, *H. influenzae* and *M. catarrhalis*). Clinical data related to age, gender, diagnosis and samples are described. *S. pneumoniae* isolates were tested against penicillin, amoxacillin, amoxacillin/clavulanic acid, cefuroxime axetil, cefaclor and azithromycin. Minimal inhibitory concentrations (MIC) were determined by Etest methodology. Interpretative criteria used were those described by NCCLS documents M100-S13. *H. influenzae* and *M. catarrhalis* isolates were tested for beta-lactamase production by chromogenic cephalosporin method [Cefinase (R)].
Results: There were 290 isolates from 258 children less than 5 years old. Most samples were from middle ear fluid (59.9%), followed by nasopharyngeal swab (23.7%) and oropharyngeal swab (12.9%). Age group of $>2 \leq 5$ years old represented 51.6%. Most were outpatients (93.4%) with diagnosis of acute otitis (45.9%), followed by acute sinusitis (8.9%) and recurrent otitis (7.4%). Among 139 isolates of *H. influenzae* and 35 isolates of *M. catarrhalis*, 13.7% and 94.3% were beta-lactamase producers, respectively. Among *S. pneumoniae* ($n = 116$), 100% were susceptible (S) to amoxicillin (MIC90 = 0.094 μg/mL), 13.0% were intermediate (I) and 1.7% resistant (R) to penicillin (MIC90 = 0.125 μg/mL), 17.2% I and 6.9% R to azithromycin (MIC90 = 1.0 μg/mL), and 0.9% I and 6.9% R to cefaclor (MIC90 = 0.75 μg/mL).
Conclusions: Significant rate of beta-lactamase production in *H. influenzae* was detected, while an expected rate was observed in *M. catarrhalis*. In *S. pneumoniae*, penicillin full resistance was still rare, although intermediate susceptibility was common. Azithromycin resistant/intermediate *S. pneumoniae* was more common than the latter in this population. Empiric therapy with penicillins alone or in low dose should be avoided in this population.

P1310 *Streptococcus agalactiae*: vaginal and rectal carriage in females in childbirth, incidence of early-onset disease, distribution of serotypes, susceptibility to antimicrobials

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Objectives: To screen females in childbirth for vaginal and rectal *Streptococcus agalactiae* (group B strains – GBS) carriage and to establish the incidence of early-onset disease (EOD) due to GBS, distribution of serotypes and GBS susceptibility to antimicrobials in the Czech Republic (CR).
Methods: Females in childbirth were screened for vaginal and anorectal carriage of GBS based on the CDC recommended criteria. Invasive strains isolated from newborns were collected from 30 microbiological and clinical centres all over the CR within prospective active surveillance for EOD. In parallel, the EOD incidence was monitored in a perinatology centre in Ceske Budejovice in passive retrospective and prospective studies. Serotypes were identified by a precipitation reaction with home-made rabbit sera and antigenic extracts prepared according to Lancefield's modification. The minimum inhibitory concentrations of penicilllin, ampicillin, cefotaxime, tetracycline, erythromycin and clindamycin were evaluated according to the NCCLS guidelines.
Results: Altogether 586 females in childbirth were investigated to show an overall colonisation rate of 29.3% (172/586) in the vagina and/or in the rectum. During a 3-year active surveillance, 141 invasive GBS isolates from newborns were collected in the reference laboratory; the incidence was calculated to be 0.96 per 1000 live births. Based on passive surveillance, the following incidence rates were documented: 1.2 per 1000 live births and 0.5 per 1000 live births prior to and after implementation of the EOD prevention project. Serotype III prevailed, followed by types Ia, II and V identically among women and neonates. All our isolates were susceptible to beta-lactam antibiotics. Resistance to erythromycin (and clindamycin) was found in 4.4% isolates from pregnant women, i.e. with an almost double frequency as compared with invasive strains isolated from neonates (8.5%). Resistance to tetracycline was found in 84.3% of the isolates from females and in 91.5% of the strains from neonates. The majority of isolates of GBS resistant to erythomycin (65.5%) belong to type V.
Conclusion: Compared with the EOD incidence, GBS carriage in pregnant woman is rather high in the CR as compared with the literature data. Our findings confirm uniform susceptibility of GBS to penicillin and other beta-lactam antibiotics tested. The study showed significance of type V strains in perinatology.

P1311 Sorbitol non-fermenting (SNF) *E. coli* strains in children with acute diarrhoea

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Introduction: Shiga toxin-producing *E. coli* strains (STEC) have been recognised as important aetiologic agents of diarrhoea and rarely serious outbreaks and sporadic cases of life-threatening haemorrhagic colitis and haemolytic uraemic syndrome (HUS) worldwide. *E. coli* O157:H7 is the most frequently identified serotype. A characteristic phenotypic feature of this pathogen is the inability to ferment sorbitol after overnight incubation. Sorbitol–MacConkey agar (SMAC) is used for routine screening.
Objective: We wanted to determine the frequency and characteristics of SNF *E. coli* strains in children with acute diarrhoea attending a general paediatric hospital in Athens, Greece, in two predetermined chronological periods.
Materials and methods: Between 1/1988 and 1/1989 as also between 1/2000 and 1/2002, 850 and 3805, respectively, faecal samples from children (3–14 years of age) with acute diarrhoea were screened in SMAC (Biomerieux). The SNF strains were identified

using API20, serotyped with poly/mono specific serum and immune chromatography. For the detection of shiga-toxin, the Shiga Toxin Micro plate Assay (ProSpecT) was used. Susceptibility tests to antibiotics were performed by disk-diffusion method according to NCCLS recommendations.
Results: Out of 4655 stool specimens 44 (0.9%) were SNF (21 strains the earlier period and 23 the latter). No O157:H7 was isolated and no shiga toxin was detected. Of these 44 strains, 7 (15.9%) matched with EPEC serotypes, O127:B8 (n4), O111:B4 (n : 2) and O55:B5 (n : 1). The SNF strains showed significant resistance to Ampicillin (36.4%), Trim/sulfa (20%) and Cephalothin (20%).
Conclusion: Our study demonstrates that O157:H7 *E. coli* strains are not detected in Greek children. In contrast EPEC were frequent among the SNF *E. coli* over a decade. We recommend culture in SMAC, antiserum serotyping and shiga toxin detection with EIA in children with acute diarrhoea.

P1312 *Rickettsia typhii* infection in children in Cyprus

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Objectives: *Ricketsia typhii* is an obligate intracellular parasite, which causes the zoonotic infection of murine typhus. This study aims at identifying clinical cases of murine typhus in children in Cyprus and study the epidemiological and clinical characteristics of the disease.
Methods: Prospective study of all cases of murine typhus occurring over a period of 2 years (October 2001 to September 2003) in children in Cyprus diagnosed and managed by primary care paediatricians or admitted in hospitals. The presence of antibodies against *R. typhii* with titres of IgG≥:960 or IgM≥:400 and/or a fourfold rise of the IgG titre between two assays were considered as a strong indication of acute infection. The minimal presumptive clinical criteria for the diagnosis were the presence of fever and/or headache and/or skin rash.
Results: During this time 15 patients fulfilled the serological and clinical criteria of the disease. Ages ranged from 4 to 14 years with mean age 8.5 years. Most cases occurred in summer and early autumn, while greatest incidence was noticed in agricultural areas. Most common clinical manifestations were fever (100%), chills (90%) and rash (55%). Lymphadenopathy was also frequent (57%) but not splenomegaly (25%). Mean duration of fever from onset until diagnosis was 7.6 days. Laboratory abnormalities included moderately elevated ESR, raised transaminase levels (72%), leukopenia (25%) and thrombocytopenia (17%). Most patients required hospitalisation mainly because of persistent fever. One of these cases was complicated with meningoencephalitis (6.6%). However, the outcome after appropriate treatment was good in all patients.
Conclusions: *R. typhii* in children in Cyprus is not a rare disease. It mainly attacks school age children who usually present with persistent fever, rash and lymphadenopathy. It usually runs a benign course and responds favourably to appropriate antibiotic treatment.

P1313 Genomovar status and antibiotic resistance of *Burkholderia cepacia* complex isolates in cystic fibrosis centre in Kosice, Slovakia

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Objectives: The aims of the study were: (i) to evaluate the prevalence of *Burkholderia cepacia* complex (Bcc) isolates in patients with cystic fibrosis (CF) (ii) to determine their genomovar status (iii) to determine antibiotic susceptibility of each Bcc isolate and (iv) to determine the most appropriate bactericidal antibiotic combination.
Methods: From August 2000 to November 2003, we collected 506 sputum samples from 42 patients with cystic fibrosis attending The Regional CF Centre in Kosice, Slovakia. All sputum samples were examined by standard laboratory culture techniques including plating on the Burkholderia Cepacia Selective Agar (BCSA). All putative Bcc isolates were further subjected to recA-based nested PCR to confirm the correct assignment to the genus Bcc and to determine the genomovar status. Antibiotic susceptibility testing for the Bcc isolates was evaluated by standard laboratory techniques using disk-diffusion method. Multiple combination bactericidal antibiotic testing for multiresistant isolates of the Bcc was done in microtitre plates using a modified time vs. kill curve method.
Results: The prevalence of Bcc isolates is 26.1% (11 patients). Six out of 42 CF patients (14.3%) have been infecting with multiresistant strains of genomovar III, recA subgroup IIIA. One patient harbours genomovar III, recA subgroup IIIB, and the remaining three positive patients have the infection with genomovar IV. The most effective single antibiotic against multiresistant strains of genomovar IIIA are meropenem and ceftazidim. The most effective triple-antibiotic combinations contain high-dose tobramycin, meropenem and the third additional antibiotic (ceftazidim, ciprofloxacin, amikacin)
Conclusion: *Burkholderia cenocepacia* (genomovar III) is a multiresistant opportunistic pathogen with the most pronounced negative effect in morbidity and mortality of CF patients. The accurate identification of Bcc completed with molecular genetic tools is very important from epidemiological point of view in order to control and minimise cross-infection in CF patients.

P1314 Late clearance of viraemia in four perinatally HCV-infected children: should the concept of HCV infection chronicity be revised in paediatrics patients?

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Background: Current definition of HCV infection in a perinatally exposed baby still relies on two positive HCV-RNA test (one during the first year of life) and/or anti-HCV positivity beyond the 18th month. The long-term outcomes of HCV infection vertically acquired are unknown and unpredictable. In paediatric setting, the progression to chronicity of HCV-related liver damage seems to be very frequent but the true liver disease is generally milder than in adults. Because of the young age of the patients, an antiretroviral treatment should be advisable, but the experience with the available drugs have been dissatisfactory. In particular, we still lack firm criteria for treating HCV infected children, and also when to start therapy still remains debated.
Patients: Based on the above criteria, between 1992 and 2003, 27 HCV vertically infected children were identified at our centres. Starting from birth, anti-HCV, HCV-RNA and ALT assessment were performed every 4 months during the first year of life and then every 4–6 months (mean follow-up 58 months, range 4–128).
Results: All the 27 babies proved to be HCV-RNA positive by the sixth month and thereafter till the first negative result. A sustained clearance of HCV-RNA could be documented in 4/27 cases (15%). In two cases (genotypes 2a/2c and 3a, respectively) the first HCV-RNA negative was observed at 18 months of age and then confirmed by three subsequent negative results obtained at 3 months intervals. In one case (genotype 2a), HCV-RNA became undetectable at 60 months of age; 4 years later the PCR test is still negative. In the last child HCV-RNA tested positive from birth to 48 months (genotype 1b); the viraemia proved to be negative by the age of 5 years and then had been steadily negative for more than 5 years. All the four children are steadily anti-HCV negative with normal ALT values.
Conclusion: Our data suggest that in children at risk for HCV infection vertically acquired, the diagnostic criteria of infection and chronicity should be reconsidered on the basis of test PCR results evaluated during a more extended period of follow-up. In case with no evidence of severe liver damage, the antiretroviral therapy should not be taken into consideration for children under 5 years of age or in any case without an adequate follow-up.