Oral presentations

Mobile resistance elements in Gram-negatives

S1 Plasmids in clinically-significant Gram-negative bacteria

A. Carattoli (Rome, IT)

Bacteria carry extrachromosomal, self-replicating genetic elements, called plasmids. Natural plasmids have systems guaranteeing their autonomous replication but also mechanisms controlling the copy-number and ensuring stable inheritance during cell division. Plasmids have evolved as an integral part of the bacterial genome, providing additional functions to their host. The best example of functions associated to a plasmid is given by the antimicrobial resistance. Resistance genes located on plasmids offer immediate advantage to their host under antimicrobial pressure and they can be easily exchanged among bacteria of different species and genera, through the natural process of conjugation, rapidly spreading in bacteria of diverse origin and source.
Plasmids are playing the major role in the diffusions of antimicrobial resistance in Gram-negative bacteria, promoting the spread of resistance genes coding β-lactamases such as CTX-M, SHV, TEM, DHA, CMY, VEB, or aminoglycoside modifying enzymes or proteins mediating quinolone resistance.
Plasmid-mediated antimicrobial resistance arises from a complex multifactorial process supported by a panoply of mobile genetic elements that contain and transfer resistance determinants. Bacterial plasmid sequence comparisons provided the historical events through which these genetic determinants are assembled by transposition, homologous recombination, and illegitimate recombinational events.
Plasmids also play a very important role in bacterial pathogenesis as carrier of virulence genetic traits. Several resistance plasmids have been described in Enterobacteriaceae to carry virulence factors, such as bacteriocins, siderophores, cytotoxins, or adhesion factors and virulence plasmids have been described to carry resistance genes. Plasmids are consequently a fashionable field of scientific research.

S2 Insertion sequences, transposons and repeated elements

T. Naas (Paris, FR)

Among the most important issues of clinical microbiology, increasing prevalence of bacterial pathogens expressing resistance to multiple antimicrobial agents is of special concern. The observed high frequency of multiresistance suggests mechanisms by which bacterial species can concentrate and efficiently exchange a variety of resistance determinants. Lateral gene transfer, which has been proposed as a fundamental process underlying bacterial diversity, is mainly mediated through plasmids and phages. While integrons are capable of concentrating resistance genes, they are uncapable of self-transposition. The mobility of the resistance genes is mediated by insertions sequences and transposons. Along with the discovery of novel resistance genes, the number of different transposable elements isolated and characterised at the nucleotide sequence level is exploding, and over 1500 different ISs have been identified to date.
Bacterial insertion sequences (IS) are small DNA segments (<2.5 kb) with a simple genetic organisation. Most IS elements exhibit short terminal inverted-repeat sequences (IR) and encode a transposase, an enzyme that is required for transposition. These mobile elements play an important role in assembling sets of "accessory" functions in bacteria (such as genes forming parts of degradative or catabolic pathways) and in dissemination of resistance genes. By inserting within a coding sequence they may inactivate the gene, or by inserting upstream of a gene they may modify its expression. ISs may also help integration of plasmids into the chromosome of bacteria. Some ISs, such as IS*Ecp1*, are capable of mobilising nearby DNA, by a mechanism similar to one-ended-transposition, while others may form a composite transposon, being capable of carrying the sequence located between the two ISs. True transposons such as Tn7, and the large group of type II transposons of the Tn3 family are complicated structures and include multiple antibiotic resistance genes carried by integrons. The association of integrons with transferable elements may promote rapid dissemination among clinical strains, and create further opportunities for inclusion of additional resistance determinants. Recently novel genetic structures, Repeated Elements (Re), have been described being capable of mobilising resistance genes through a transposition/recombination process that still needs to be fully understood.
The study of novel resistance mechanisms and their underlying genetic background revealed novel mobile DNA elements responsible for their mobilisation. These elements are of interest for clinical microbiology, since they are associated to the emergence of novel antibiotic resistance genes, and for fundamental research, since novel genetic mechanisms for gene mobilisation and dissemination were identified.

S3 The two mechanisms of gene capture behind most trimethoprim resistance in enteric bacteria

L. Sundström (Uppsala, SE)

Synthetic antibacterial drugs such as antifolates, sulfonamides and quinolones have been widely used in human and veterinary practice over the last 60 years. The clinical efficacy of these drugs is undermined by the integration and spreading of resistance borne on transmissible plasmids and genetic islands. In 1977, Pattishall et al. divided the plasmid-borne trimethoprim resistance genes into two distinctive structural classes later referred to as dfrA and dfrB. The number of identified gene types has grown to more than 30 at present. We matched the contexts of all sequenced dfr genes with signatures of two gene capture mechanisms. These are uptake of gene cassettes in integrons by site-specific recombination and one-ended transposition mediated by IS91-like elements referred to as common regions or ISCR. The data we assembled showed that trimethoprim resistance in Gram-negative bacteria is spread, at nearly full coverage, via either of these recombinational paths. Nineteen types of dfr genes in three families, examplified by the gene types A1, A12 and B1, are borne on integron-based cassettes while seven dfr gene types, A3b. A9, A10, A19, A20, A23 and A26 (Grape et al., unpublished) have been inserted via the second recombination path. IS91 family insertion elements use a one-ended transpositional mechanism to produce single-stranded DNA through rolling circle replication. Integron cassettes use recombination sites that are in fact recognized by the cognate tyrosine recombinase in the form of harpins. This suggests that IS91 might enhance excision and integration of cassettes that are situated within replication range of IS91-like elements. If the two gene capture systems are indeed engaged in a functional crosstalk this could explain their frequent co-localisation. The spreading of resistance to other synthetic drugs differs. For instance, sulfonamide resistance in Gram-negative bacteria is ubiquitous but much less diverse with only three gene types in the metagenomic pool. The recombinational spread of sul genes is cryptic and may be diverse. However, one of the genes, sul1, is strongly linked with both integrons and common regions of the IS91 type. Sulfonamides were introduced prior to other modern antibacterial drugs suggesting that it was the key selecting factor for making the dual recombinational platform of integrons and IS91-like elements fixed in most bacterial populations. Quinolone resistance gets contribution from transmissible qnr alleles of three families reported in connection with IS91-like regions (Robicscek et al. 2006).

S4 Common region as a source of spread and expression of antibiotic resistance genes

T. Walsh (Cardiff, UK)

Bacteria are noteworthy for their remarkable genetic plasticity responding and adapting to environmental changes that often as not involves the exchanging of DNA molecules in the same cell and between bacterial cells. Insertions sequence common regions (ISCRs) were first discovered and reported in the early 1990's as a DNA sequence of 2,154 bp that was found in two complex class 1 integrons, In6 and In7. The sequence was initially described as a common region (CR) to distinguish it from the 5′ and 3′ conserved sequence of class 1 integrons. ISCRs were also interesting in that they are intimately associated with different resistance genes carried on each integron. ISCR elements have been found in many Gram-negative bacteria and have been associated with an array of different antibiotic resistant functions including qnr, ESBLs, AmpC β-lactamases, metallo-β-lactamases, aminoglycoside and chloramphenicol resistance, dhfr and sul genes. Some bacteria contain several copies of these elements that have probably occurred via replication followed by homologous recombination. They can be located on either the chromosome or/and plasmid.

There are now at least 15 known types of ISCR elements that when aligned share key amino acid motifs with each other and that of IS91. These motifs have been shown to be involved in DNA recognition and mobility. ISCR elements are very unusual in that, like IS91, they can mobilise large sections of adjacent DNA via a rolling circle replication. Normally, termination of replication occurs at a defined site; misreading of this site leads to replication of large sections of DNA to the left-hand end of the element including antibiotic resistance genes. Many elements are found next to class 1 integrons which we believe to be expressed and active – possibly driven by a hybrid promoter. The only ISCR element shown not to be expressed is ISCR2. They are now commonly found in Enterobacteriaceae, Gram-negative non-fermentors and occasionally Gram-positive bacteria and have been found in clinical isolates from every continent. Their impact on the spread of antibiotic resistance genes is only now beginning to be understood.

Diagnosis and treatment of septic shock (Symposium arranged with the International Sepsis Forum)

S5 The innate immune response to *P. aeruginosa* in human monocytes is mediated mainly by TLR-2 and mannose receptor

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P. aeruginosa is a human pathogen that causes life threatening infections in immunocompromised host. We have previously shown that Extracellular Slime Glycoliprotein (GLP) produced by all mucoid and non mucoid strains is a potent activator of NFkb transcriptional activity and TNF-α induction in fresh human monocytes.

Objectives: To examine which innate immune receptors are involved in signaling proinflammatory cytokine production induced by *P. aeruginosa*.

Methods: We stimulated for 6–24 h fresh human monocytes either with *P. aeruginosa* Slime GLP (100 μg/mL) or viable bacteria (MOI 10:1) in the presence or absence of specific blocking antibodies against TLR4, TLR2 and Mannose receptor (10 μg/mL). Proinflammatory cytokine production (IL-1b, IL-6, TNF-α) was measured in the culture supernatants by ELISA. Phagocytosis was inhibited by the addition of Cytochalasin D (1 μg/mL) 30 min before stimulation. Receptor mediated activation of NFkb was examined by cotransfection experiments, using human embryonic kidney (HEK)-293 cells and plasmids encoding mannose receptor, TLR4 and TLR2–CD14 complex along with NFkb reporter driving the expression of luciferase gene. NFkb activation was detected by measuring luciferase activity.

Results: Blocking of phagocytosis or mannose receptor function resulted in 90% inhibition of proinflammatory cytokine production. TLR2 blocking resulted in 70–80% inhibition whereas TLR4 blocking had less prominent effect (30–40%). Challenging of HEK-293 cells expressing only mannose receptor with Slime GLP or viable bacteria resulted in NFkb activation by 6–10 fold and 20–30 fold correspondingly. Co expression of TLR2 in the same cells increased the NFkb activation by 3 fold in both ways of stimulation, whereas coexpession of TLR4 resulted in just detectable increase, 1.2–1.5 fold.

Conclusion: Our results suggest that mannose receptor acts not only as phagocytosis receptor for *P. aeruginosa*, but also as signaling receptor and cooperates with TLR2 for maximum NFkb activation and proinflammatoty cytokine production. These findings provide a new insight for future development of new immunotherapies.

Challenges in pharmacokinetics and pharmacodynamics

S11 PK/PD in critically ill patients

F. Scaglione (Milan, IT)

Antimicrobial treatment of infections in critically ill patients remains a significant challenge with persisting high mortality and morbidity. Early and appropriate antibacterial therapy remains an important intervention for such patients. To optimise antibacterial therapy, the clinician should possess not only knowledge of the pharmacokinetic and pharmacodynamic properties of commonly used antimicrobials, but also how these parameters may be affected by the pathophysiological changes occurring during sepsis or septic shock.

Pathophysiological changes can be contrasting. Sepsis, and the non-antibiotic treatment, increasing renal preload may result in higher antibacterial clearances. Alternatively, sepsis can induce multiple organ dysfunction, including renal and/or hepatic dysfunction, causing a decrease in antibacterial clearance.

Aminoglycosides, β-lactams and carbapenems are distributed in plasma and interstitial space, therefore their concentrations could be dramatically affected during sepsis. These drugs can have a higher Vd during sepsis leading to a reduced Cmax. Some patients with serum creatinine within the normal range can have higher than normal drug clearances, thereby producing low serum concentrations. If a drug needs to have a minimum serum concentration maintained (e.g. β-lactams), a high drug clearance will lead to underdosage for renally excreted drugs. All β-lactams should, in such patients, be administered more frequently than suggested in non-sepsis patients; administration by continuous infusion should be considered. In relation to the aminoglycosides, this means that not only are large doses required to be administered, but because of a high CLCR these antibacterials may also need dosing even more frequently than every 24 hours.

Regarding the renal clearance of the fluoroquinolones, in the presence of a high CLCR can be assumed that fluoroquinolone clearance is also high. If this were true these antibacterials would also need to have higher daily doses than proposed in the standard literature.

In conclusion the treatment of infections in critical ill patients remains a significant challenge given the persisting high mortality rates. Data suggest that effective antibacterial therapy remains the most important intervention available to the clinician. In treating sepsis, a clinician must be aware of the impact of the various pathophysiological and subsequent pharmacokinetic changes that can occur during sepsis.

Infection control

O14 Surgical site infection surveillance in France: the first 1999–2004 trend analysis

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Background: Surgical site infection (SSI) is one of the most frequent nosocomial infections. Since 1999, a national coordination of regional networks has been implemented to gather SSI incidence data according to standardised method. The aim of the current study was to describe the largest database ever collected in France on SSI and to analyse a 6-year temporal trend.
Methods: Surgery patients were enrolled by voluntary participating wards in a yearly 3-month incidence survey. In each ward, 200 consecutive surgery procedures should be included and patients followed up to 30 days after surgery. SSI was defined based on standard CDC criteria. For each patient, risk factors were collected on the day of surgery including age, ASA score, Altemeier wound class, type and duration of procedure, emergency/elective, and when videoscopy surgery was performed.
Results: During 6 years, the study included 620,176 operations (17,430,253 operated patient–days follow-up; median post-operative follow-up: 28 days). The overall SSI incidence rate was 1.68%. Organ space and deep incisional SSI accounted for 41.7% although their proportion varied according to the type of surgery. SSI incidence rate increased from 0.91% [0.88–0.94%] for NNIS-0 patients to 13.8% [12.5–15.2%] for NNIS-2, 3 patients. The SSI incidence varied from 1.15% for herniorraphy to 9.2% for colon surgery. In NNIS-0 patients, emergency surgery increased the SSI risk for C-section whereas videoscopy surgery was at lower risk for cholecystectomy. From 1999 to 2004, NNIS-0 SSI incidence decreased from 1.1 to 0.9 for 100 operated patients (relative difference: −18%). According to procedure, the trend remained significant only for herniorraphy.
Conclusion: This database provided thorough standardised estimate of SSI incidence according to various surgery procedures. Impact of the national policy on SSI incidence remains to be further evaluated, although encouraging results were evidenced for specific surgery.

O15 Changes in healthcare-associated infection rates in French maternity units from 1997 to 2003

A. Vincent, L. Ayzac, R. Girard, E. Caillat-Vallet, C. Chapuis, A.M. Dumas, C. Gignoux, C. Haond, C. Launay, F. Tissot-Guerraz, J. Fabry and surveillance networks' units

Objectives: Healthcare associated infection (HAI) incidence rates after delivery range from 0.26% to 20.3% according to the mode of delivery, the maternity activity, women risk factors. Data on HAI surveillance in maternity units are lacking. The Mater Sud-Est Study Group is a HAI continuous surveillance network on maternity units located in south eastern France. We report changes in risk-adjusted HAI rates over a 6-year long surveillance period in this maternity units network.
Methods: 161,077 vaginal deliveries and 37,074 cesarean deliveries were included in the surveillance between January 1st 1997 and December 31st 2003. We studied the changes in four HAI: endometritis and Urinary tract infection (UTI) after vaginal deliveries, surgical site infection (SSI) and UTI after cesarean deliveries. We used a logistic regression modeling to estimate risk-adjusted HAI rates. The year of delivery was considered as a risk factor. The trend of risk-adjusted HAI rates over the study period was studied by a linear regression of the year-of-delivery odds ratios for each targeted HAI.
Results: The rate of endometritis and UTI after vaginal deliveries was 0.3% (534/161,077) and 0.5% (728/161,077) respectively. Over the study period the decrease in endometritis odd ratios was statistically significant. We found no statistically significant trend in vaginal delivery's UTI. The rate of SSI and UTI after cesarean deliveries was 1.5% (571/37,074) and 1.8% (685/37,074) respectively. Over the study period the decrease in SSI and UTI odd ratios was statistically significant.
Conclusion: These findings highlight the positive effect of participating in a surveillance network for infection control and for improvement of care.

O16 Bacterial population kinetics on hands during two consecutive surgical hand disinfection procedures

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In Europe, the efficacy of surgical hand antiseptics is investigated only for a single application. In clinical practice, however, consecutive applications are common but the effect on the bacterial density on hands is largely unknown. We therefore studied the effect of different consecutive applications on the resident bacterial flora on hands.
A propanol-based hand rub (Sterillium®, based on 45% propan-2-ol, 30% propan-1-ol and 0.2% mecetronium etilsulfate) and the reference alcohol of EN 12791 (60% n-propanol) were tested in two consecutive applications with 3 h between them. Four variations were tested. The first application of Sterillium was always for 1.5 min, the second application was for 1.5, 1 or 0.5 min. The reference alcohol was applied for 3 min (both applications). The efficacy of the three product variations was compared to the reference treatment of EN 12791. All experiments were performed in a Latin-square design with 20 volunteers. Pre- and post-values (immediately and 3 hr after each applications) were obtained according to EN 12791.
The first reference disinfection (3 min) reduced the bacterial density by 2.87 ± 1.00 $\log_{10}$-steps (immediate efficacy) and 2.27 ± 1.25 $\log_{10}$-steps (after 3 h). Sterillium applied for 1.5 min yielded a similar reduction at both sampling times. Immediately after the second 3 min reference disinfection bacterial density was reduced by 0.45 ± 1.05 $\log_{10}$-steps. Application of Sterillium yielded larger reductions with 0.71 ± 1.32 (0.5 min application time), 0.79 ± 1.63 (1 min application time), and 1.12 ± 1.04 $\log_{10}$-steps (1.5 min application time). The difference between the four treatments, however, was not significant ($p = 0.089$). After 3 h under the surgical glove bacterial density further decreased with 1.11 ± 1.04 $\log_{10}$-steps for the reference disinfection. A 1 min application of Sterillium yielded the largest reduction (1.89 ± 1.02) followed by a 1.5 min treatment (1.67 ± 0.98) and a 0.5 min treatment (1.08 ± 0.86). There was a significant difference between all four treatments ($p = 0.005$; Friedman test) but none of the short treatments with Sterillium was significantly less effective than the 3 min reference treatment ($p > 0.05$; Wilcoxon-Wilcox test).
Overall, a simple 1.5 min application of a well-formulated propanol-based hand rub for surgical hand disinfection keeps the bacterial density as low as possible even in two consecutive surgical procedures of 3 h.

O17 Surveillance of occupational blood and body fluids exposures

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Background: National surveillance of occupational blood and body fluids exposures (BBFE) in France is conducted since 2002 through the Nosocomial Infection Early Warning, Investigation and Surveillance Network (Raisin) in collaboration with Geres (Groupe d'Etude sur le Risque d'Exposition des Soignants aux Agents Infectieux).
Methods: Participation of healthcare facilities (HCF) is voluntary and anonymous. BBFE occurring from 01/01/04 to 31/12/04 were documented using a standardised questionnaire documenting the nature, circumstances (mechanism, type of device, infectious status of the source) and follow-up of each BBFE. Incidence of BBFE is reported per 100 hospitalisation beds, by type of personnel per 100 full time equivalents (FTE), or by type of material per 100,000 devices.

Results: In 2004, 13,041 BBFE were documented in 371 participating HCF, which accounted for 15% of HCF and 29% of hospitalisation beds in France. BBFE overall incidence was 8.9 per 100 beds. Considering that all French hospitals account for 465,494 beds, 41,276 [95% CI: 40,896–41,656] BBFE could have occurred in France in 2004. HCV or HIV status of the source was not known for more than 20% of documented BBFE. Post-exposure prophylaxis (PEP) was prescribed to 4.5% of exposed personnel (vs. 5.8% in 2003 and 6.3% in 2002); this decrease may reflect the impact of April 2003 French recommendations, which reduced PEP indications. For the first time in 2004, sutures were the most frequent cause of BBFE associated with needles (more than subcutaneous injections) and accounted for 1,103 (11%) of all BBFE; one third occurred among residents, and 20% in ICU or emergency rooms (beyond surgery or obstetrics). Prevention through education and use of safety devices (such as blunt suture needles) may thus be a priority. Data from a cohort of 173 HCF which participated in 2003 and 2004 also were compared and demonstrate significant progresses. Compliance to glove use increased from 58.6% in 2003 to 62.3% in 2004, and BBFE incidence among nurse assistants fell from 2.3 in 2003 to 2.1 per 100 FTE in 2004. Last, BBFE incidence fell from 17.2 to 13.7 per 100,000 catheters, and from 71.6 to 43.2 per 100,000 implantable venous access systems.
Conclusion: AES-Raisin is one of the biggest BBFE surveillance network and results demonstrate an increase in observance to standard precautions and a significant decrease in the incidence of some types of BBFE. They also point out future priorities for improvement.

O18 A randomised trial of 2% chlorhexidine in 70% alcohol compared with 10% povidone-iodine for venipuncture site disinfection: effects on blood culture contamination rates

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Background: Contaminated blood cultures have been recognized as a bothersome issue for decades, and continue to cause a frustration for clinicians. The contamination rates vary widely between institutions from less than 1% to over 6%. Skin antiseptics can prevent the contamination of blood cultures. To our knowledge, no randomised trial of 2% chlorhexidine in 70% alcohol for venipuncture site disinfection has been conducted.
Objective: Our study was aimed to evaluate the efficacy of venipuncture site disinfection with 2% chlorhexidine in 70% alcohol compared with 10% povidone-iodine in preventing blood culture contamination.
Patients and Methods: A prospectively randomised investigator-blinded trial was conducted in all patients hospitalised in Internal Medicine wards and attended at emergency department at King Chulalongkorn Memorial Hospital, Bangkok, Thailand from August 15 to October 31, 2006. Antecubital venipuncture sites were randomly disinfected with either 2% chlorhexidine in 70% alcohol or 10% povidone-iodine, and blood cultures were drawn by medical students or residents. The blood culture contamination rate associated with each antiseptic was then determined.
Results: Of 2,146 blood culture collected during the study, 108 (5.03%) were contaminated with skin flora. The contamination rate for blood cultures after 2% chlorhexidine in 70% alcohol was 3.2% (34 of 1,068), compared with a rate of 6.9% (74 of 1,078) ($P < 0.001$) after 10% povidone-iodine. Of the inpatient wards, the contamination rate was 2.6% (18 of 695) and 3.9% (28 of 709) after 2% chlorhexidine in 70% alcohol and 10% povidone-iodine, respectively ($P = 0.013$). Of emergency department, the contamination rate was 4.3% (16 of 373) and 12.5% (46 of 369) after 2% chlorhexidine in 70% alcohol and 10% povidone-iodine, respectively ($P < 0.001$). The most common contaminant organism was coagulase-negative staphylococci (81%).
Conclusion: 2% chlorhexidine in 70% alcohol is superior to 10% povidone-iodine for venipuncture site disinfection before blood culture sampling.

O19 Assessing the biological efficacy and rate of recontamination following hydrogen peroxide vapour decontamination

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Objectives: The inanimate hospital environment can become contaminated with nosocomial pathogens. Hydrogen peroxide vapour (HPV) decontamination has proven effective for the eradication of persistent environmental contamination but the rate of recontamination following HPV decontamination is largely unknown. We investigated the extent of methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE) and gentamicin-resistant Gram-negative rod (GNR) contamination in a ward side-room occupied by a patient with a history of MRSA, VRE and GNR infection and colonisation.
Methods: Fifteen standardised sites in the room were sampled using a selective broth enrichment protocol to culture for MRSA, VRE and GNR. Sampling was carried out before cleaning, after cleaning, after HPV decontamination and at intervals over the subsequent 19 days on two separate occasions.
Results: Environmental contamination was identified before cleaning on 60%, 30% and 6.7% of sites for MRSA, GNR and VRE, respectively and 40%, 10% and 6.7% of sites after cleaning (figure). Only one site (3.3%) was contaminated with MRSA after HPV decontamination (figure). No recontamination with VRE was identified and no recontamination with MRSA and GNR was identified in the two days following HPV decontamination (figure). Substantial recontamination towards pre-cleaning levels was identified by day five and six after HPV decontamination for MRSA. Recontamination with GNR at approximately post-cleaning levels was noted on days 7, 8 and 19 (Figure 1).

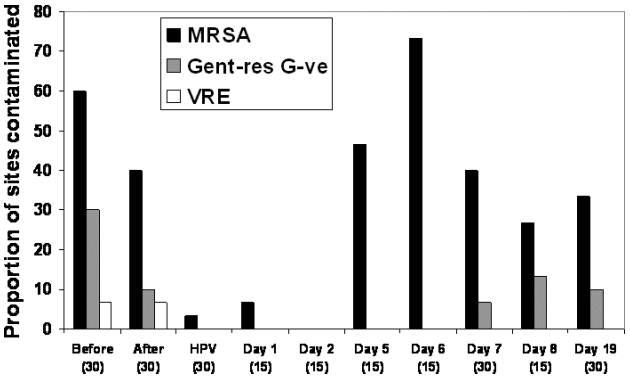

Fig. 1. Proportion of sites contaminated with MRSA, gentamicin-resistant Gram-negative bacteria and VRE before cleaning, after cleaning, after hydrogen peroxide vapour (HPV) decontamination and at intervals over the subsequent 19 days during two experiments in a ward side room. (Number of site sampled in parentheses.)

Conclusions: HPV is more effective than standard terminal cleaning for the eradication of nosocomial pathogens. Recontamination was not immediate for MRSA and GNR but returned towards pre-cleaning levels for MRSA and post-cleaning levels for GNR within a week in a room occupied by a patient colonised with MRSA and GNR. This finding has important implications for the optimal deployment of HPV decontamination in hospitals.

O20 Evaluation of the knowledge of hospital cleaning staff about prevention of nosocomial infections

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Objectives: Increasing morbidity and mortality due to nosocomial infections (NI) necessitates the more stringent implementation of

infection control measures. Detection of risk groups and infection sources and knowing the transmission ways of infections are important for the prevention from NI. In this study it was aimed to evaluate the knowledge level and behaviour models of hospital cleaning staff about NI in our setting which is a 1788 bedded tertiary care educational hospital.
Methods: A questionnaire of with 21 questions was implemented to the hospital cleaning staff, who volunteered to enter the study. The questionnaire was composed of two parts: first part contained parameters for determination of sociodemographic properties and the second contained questions about evaluation of the knowledge about prevention from NI. Questions were prepared by using the references about the subject and by the help of the executives of the cleaning staff firm and statistics unit. Data were evaluated by SPSS 13.0 programme using Chi square and Student's t tests. The questionnaire was completed by one by one interview method.
Results: A total of 240 of 290 (82.7% of total, 122 male, 118 female, aged 36.2±8.7) hospital cleaning staff volunteered to enter the study. When evaluated according to the educational status; 55.4% were graduated from primary school and only 54% had been working in the hospital more than three years. Mean knowledge level was 18.15±3.97 (maximum 24). Knowledge level was not associated with gender, educational status and duration of working as cleaning staff ($p > 0.05$) but mean knowledge level of the staff working in the clinics was found higher than staff working in administrative sections ($p < 0.05$). 71.3% had received a formal education about prevention from NI before starting working but their mean knowledge level was not different from the others ($p = 0.294$). Only 48% and 50% knew the true order (x,y), while cleaning the patient rooms. 58.8% thought that they could prevent themselves from NI by hand washing before and after cleaning process, 80.8% stated they obeyed handwashing rules and 90.4% stated that they used gloves. Only 48.3% stated that they dried their hands by paper towels.
Conclusion: Measurement of the level of the knowledge of the hospital cleaning staff may be beneficial for determination of the existing problems. Periodical well-established educational programmes should be started to improve the current situation.

O21 Evaluation of a rapid molecular dipstick assay for the direct detection of methicillin-resistant *Staphylococcus aureus* in clinical specimens

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Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) causes increasing healthcare problems worldwide. Rapid and sensitive screening methods for direct detection of MRSA are essential to limit further spread in the hospital. The purpose of this study was to evaluate the new molecular dipstick assay GenoQuick® MRSA (Hain Lifescience, Nehren, Germany) for the direct and specific detection of MRSA in clinical specimens.
Methods: The analytical specificity of the assay was evaluated by using a subset of 25 MRSA isolates (including SCCmec types I–V); 17 methicillin-susceptible *S. aureus* (MSSA) and 38 coagulase-negative staphylococci (CoNS) of different culture collections. The lower detection limit of the assay was determined by serial dilutions of MRSA strains representing SCCmec types I to IV.
The test was evaluated for direct detection of MRSA in clinical swab specimens. MRSA carriage was analysed by both the standard culture methods [Chromagar MRSA (Becton Dickinson, Heidelberg, Germany), Columbia blood agar, trypticase soy broth] and two PCR assays [GenoQuick MRSA and GenoType® MRSA Direct (Hain Lifescience)]. Both PCR assays were performed directly from the swab and after overnight incubation in trypticase soy broth. MRSA isolates were confirmed using a mecA gene and *S. aureus* specific PCR. Susceptibility testing was performed with an automated system (VITEK 2, bioMérieux, Nürtingen, Germany).
Results: The lower detection limit of 25 CFU was determined with serially diluted MRSA strains. For analytical specificity all MRSA strains representing SCCmec types I to V were tested positive by the assay. The MSSA and CoNS were tested negative, respectively.
Of 187 patient specimens tested for clinical evaluation, 24 were identified MRSA-positive by culture and by both PCR assays. One specimen was positive only by PCR. Among the 163 culture-negative specimens, 162 were negative with both PCR assays.
The GenoQuick assay showed a diagnostic sensitivity of 100%, and a diagnostic specificity of 99.4%, a positive predictive value of 96% and a negative predictive value of 100%. Time-to-result for the direct detection of MRSA from clinial specimens is reduced to 2h 20min with the molecular GenoQuick MRSA dipstick assay (2h 5min for amplification and 15 min for detection).
Conclusions: The GenoQuick MRSA dip stick assay proved to be a rapid, sensitive and specific assay for direct detection of MRSA in clinical swab specimens in 2h 20min.

O22 Throat swabs are necessary to reliably detect carriers of *Staphylococcus aureus*

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Background: The anterior nares are considered to be the primary colonisation site of *Staphylococcus aureus* (*S. aureus*) and approximately 30% of healthy people carry the bacteria in their anterior nares. However, recent studies indicate that the throat may be an additional important site of colonisation (Nilsson P. J Clin Microbiol 2006). Most screening programmes for *S. aureus* including methicillin-resistant *S. aureus* (MRSA) require a swab from the nose only, and a swab of the throat is not considered as standard.
Objectives: To determine the frequency of positive *S. aureus* cultures with positive samples from the throat and negative from the nares.
Methods: Specimens were obtained with a sterile polyester fiber-tipped swab moistened with sterile saline from the anterior nares (5 rotations in each anterior nostril), the posterior wall of the pharynx, and the soft palate. Swabs were transported to the laboratory in a transport tube (M40 Transystem, Copan, Brescia, Italy) and put in selective enrichment broth (Chapman broth containing brain heart infusion broth with 6% NaCl, Biomedics, Madrid, Spain).
Results: A total of 905 individuals were screened for *S. aureus* between 2000 and 2005. Complete data were unavailable from 54 individuals who were excluded. Overall, *S. aureus* was isolated in 386/851 (45.4%) individuals from any site.

Screening results		No. of	% of overall
Nares	Throat	individuals	positive
pos	pos	196	50.8%
pos	neg	119	30.8%
neg	pos	71	18.4%
		386	100%
neg	neg	465	

Conclusion: Limiting *S. aureus* screening to the nares fails to identify 18.4% of carriers. Additional cost can be avoided by pooling the specimens while maintaining the higher sensitivity. Therefore, optimal screening for *S. aureus* should include swabs from both the nares and the throat. This may be even more important if screening is focused on MRSA carriage.

O23 Impact of hypochlorite disinfection on MRSA rates

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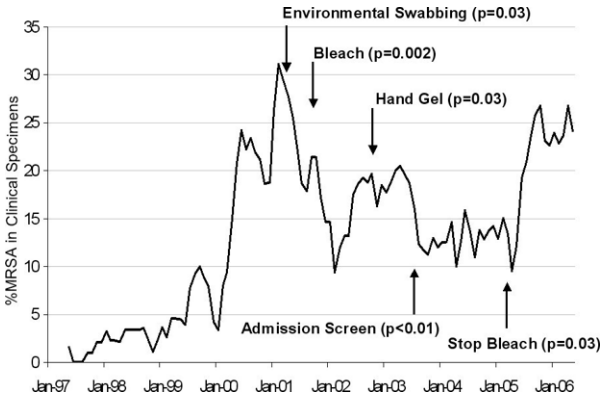
Objectives: MRSA rates in a hospital in the North-East of Scotland were significantly declining due to a series of infection control interventions.

These included terminal disinfection of the environment in isolation rooms and cohort areas by application of 1:1000 sodium hypochlorite in place of detergent (ECCMID '06, abstract P1333). We evaluated the effect of replacing sodium hypochlorite with a standard detergent.
Methods: From January 1997 to May 2006, monthly percentage, non-duplicate *S. aureus* clinical cases caused by MRSA were collated. In February 2005 hypochlorite cleaning solution as replaced by a standard detergent. Other infection control measures remained unchanged. Dynamic regression analysis with linear transfer functions and interrupted time-series analyses were used to estimate the effect to the intervention.
Results: Previously, MRSA rates wee successfully reduced due to environmental screening ($p = 0.03$), use of hypochlorite for environmental disinfection ($p = 0.002$), use of alcohol based hand disinfection ($p = 0.03$) and patient admission screening ($p < 0.01$). Stopping the hypochlorite disinfection was associated with a sudden increase in clinical cases of MRSA from 10 to 25% over a 6 month period ($p = 0.03$), with levels approaching those seen prior to the start of the infection control programme in 2001 (see figure).
Conclusions: Stopping hypochlorite environmental disinfection was strongly associated with an increase in clinical MRSA cases. This work adds significantly to the meagre published evidence that environmental contamination is important in the spread of MRSA.



Resistance surveillance

O24 Multi-drug resistant enterococci among Portuguese swine after growth promoter ban

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Objectives: To determine antibiotic-resistant enterococci in Portuguese piggery samples and to analyse antibiotic resistance genes among these strains after European growth promoter ban.
Methods: Samples from waste treatment and dry faeces from 2 pig farms in the South of Portugal were studied during 2006. Samples were plated onto selective Slanetz-Bartley agar with or without antibiotics. Bacterial identification was performed by both standard biochemical profiles and amplification of species specific genes. Antibiotic susceptibility (12 antibiotics) was determined by disk diffusion method (CLSI). Detection of genes coding for resistance [vanA, B, C1, C2, ermA, B, C, tetM, L, O, K, S, aac(6′)-Ie-aph(2″)-Ia, aph(2″)-Ib,Ic,Id, aph(3′)-IIIa, vat E] were searched by PCR.
Results: We identified 84 enterococci (9 *E. faecalis*, 17 *E. faecium*, 1 *E. gallinarum*, 2 *E. casseliflavus* and 55 *Enterococcus* spp.). Most isolates showed decreased susceptibility to tetracycline, minocycline, erythromycin, and quinupristin-dalfopristin (95%, 94%, 80%, 54%, respectively), and to a lesser extent to high-level of resistance (HLR) of streptomycin, nitrofurantoin, ciprofloxacin, HLR to gentamicin, chloramphenicol and ampicillin (52%, 33%, 32%, 21%, 11%, 10%). All were susceptible to glycopeptides. Non-susceptible isolates to tetracyclines, aminoglycosides and macrolides contained tetM (55%), tetL (54%), tetM+tetL (32%), tetS (5%), aac(6′)-Ie-aph(2″)-Ia (89%), aac(6′)-Ie-aph(2″)-Ia+aph(3′)-IIIa (47%), ermB (49%). vanC1 was linked to *E. gallinarum*, and vanC2 to *E. casseliflavus* as expected.
Conclusions: Although all growth promoters were progressively removed from EU in the course of the last 10 years, antibiotic multi-resistant enterococci were isolated in Portuguese piggeries. Whether persistence of these antibiotic resistant strains is due to selection by antibiotics or other agents deserves further studies.

O25 Experiences and results from the surveillance programme of resistance in feed, food and animals in Norway (NORM-VET) 2000–2005

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Objectives: The monitoring programme for antimicrobial resistance in the veterinary and food production sectors (NORM-VET) was established in 2000. The goals of the programme are to monitor the antimicrobial resistance situation in feed, food and animals over time, in relation to the human situation and to the resistance situation in other countries. Data from NORM-VET could form a basis for risk assessments and be a tool for targeting interventions and further to evaluate the effectiveness of such interventions. This study was performed to summarise the experiences and results obtained during the first six years of the programme.
Methods: The zoonotic agents *Salmonella* (from feed, animals and food) and *Campylobacter jejuni* (from broiler and broiler meat) were monitored annually. *E. coli* and *Enterococcus* spp. (indicator bacteria) were sampled from various animal species and meat products biannually. Specific clinical isolates from the routine diagnostic have been included biannually. The isolates have mainly been tested using a microdilution technique (VetMICTM). The minimum inhibitory concentrations were recorded and analysed in WHONET 5.3. For the categorising of the isolates as resistant or susceptible epidemiological cut-off values were applied.
Results: The occurrence of resistance in the monitored species and products is in an international perspective low and the results from the first six years of the programme show that the situation is stable.
Conclusion: Evaluation of the first six years of the programme has recognized that the relatively low number of isolates of each species and source included complicates the conclusions possible to draw from the data, especially evaluating trends over time. Even though the run costs of the programme has been limited to a minimum, it is still useful for the purpose of monitoring antimicrobial resistance within a country as Norway, where the resistance problem in the animal and food sectors still is at a very low level. It also consists as valuable source for further research of antimicrobial resistance mechanisms and development. However, the use of this source to perform risk assessments is limited as there still is a lack of even more specific data as for instance data on usage at animal or farm level.

O26 Comparison of antibiotic susceptibilities of *Staphylococcus aureus* and *S. intermedius* isolates from dog owners and their dogs

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Objectives: Antibiotic resistance in veterinary isolates has been reported to be higher than in human isolates due to frequent empirical use, and there are concerns about transfer of resistance between staphylococcal species. *S. intermedius* is the more common colonising species in dogs, but *S. aureus*, including MRSA, may also be present. Case reports suggest there can be cross-infection between companion animals and man. Increasing concern about MRSA in the community has led to recommendations for surveillance of antibiotic resistance in isolates from companion animals. This study compared antibiotic resistance in isolates of *S. aureus* and *S. intermedius* from dogs and their owners.
Methods: A cross-sectional study of owners and their dogs was performed using a convenience sample of 800 pairs recruited at six veterinary practices. Nasal swabs were collected from both owner and

dog, and held at 4ºC in transport medium until culture within 8 h of collection. Subjects completed a questionnaire providing demographic information of owner and dog, and stating if the dog had received antibiotics within the last 3 months.
Swabs were inoculated onto blood agar and mannitol salt agar and placed in 5% salt meat broth for enrichment. *S. aureus* or *S. intermedius* were identified by means of coagulase, VP, polymyxin susceptibility, and trehalose fermentation. Several colonies of each isolate were tested for susceptibility to methicillin. Antibiotic susceptibilities were determined by disc diffusion and interpreted using CLSI guidelines.
Results: *S. aureus*: 168 owners (25%) and 64 dogs (8.5%) were colonised. 16 owners and their dogs were concurrently colonised. 6 dogs (1.3%) and 4 humans (0.5%) were colonised with MRSA. Resistance to oxacillin, clindamycin, gentamicin, tetracycline and fusidic acid was significantly higher in dog isolates.
S. intermedius: 64 dogs (7.9%) and 8 owners (1.1%) carried *S. intermedius*. Four colonised owners had colonised dogs. Methicillin resistance was not detected. Resistance to chloramphenicol, clindamycin, tetracycline, and cotrimoxazole was higher in dog isolates. Resistance to fluoroquinolones and gentamicin was only displayed by dog isolates.
Conclusions: Methicillin resistance was found only in *S. aureus*, but resistance to other antibiotics was higher in *S. intermedius*. Dog isolates were more resistant than human for both species. Veterinary use of antibiotics may increase resistance and the risk of transmission of resistant strains.

O27 European Antimicrobial Susceptibility Surveillance in Animals (EASSA): Results (2002/2003) for enteric bacteria from healthy cattle, pigs and chickens from 8 countries

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Objectives: Antimicrobial susceptibility to human-use antibiotics was investigated among the commensal bacteria *E. coli* (Ec) and *Enterococcus* spp. (Ent) from healthy food animals at slaughter across the EU.
Methods: Colon or caecal content was randomly collected at 4 abattoirs per country (n = 5 per host). Each herd/flock was sampled once. Ec and Ent were isolated using standard methods. Antibiotic susceptibility testing was done by agar dilution (CLSI, M31-A2) against 9 (Ec) and 5 (Ent) antibiotics in a central laboratory. Resistance (CLSI, M100-S16) was assessed per drug/organism/country.
Results: A total 1465 Ec were recovered (cattle n = 490, pigs n = 494, chickens n = 481). Mean resistance (%) for Ec was: ampicillin (A) 3, 29, 53; cefepime 0, 0, 0; cefotaxime 0, 0, 0.4; ciprofloxacin 1, 0.4, 6; chloramphenicol 2, 16, 15; colistin 0, 0.4, 0; gentamicin (G) 1, 2, 2; tetracycline 8, 66, 65; and trimethoprim-sulfamethoxazole 4, 42, 52 respectively. For Ec, Italy and Spain consistently showed the highest resistance; Denmark showed the lowest. A total of 718 Ent isolates were recovered, comprising 356 *E. faecium*, 83 *E. faecalis* and 279 other species including *E. durans*, *E. hirae* and *E. casseliflavus*. All Ent but one bovine isolate, were susceptible to linezolid. For *E. faecium* resistance to A and G was 0–2%; vancomycin (V) resistance amounted to 1.9 to 3.5%, whereas resistance to quinupristin/dalfopristin (Q/D) combination varied: 8% (cattle), 19% (pigs) and 20% (chickens). Though low prevalence of *E. faecalis* limited conclusions, particularly in chickens (n = 6), G and V resistance was low in cattle and pigs (0–11%), resistance to Q/D was very high (46 and 83%, respectively). In the other Ent species, resistance among the 3 hosts to G and V was low (0–1.6%). Resistance to A was absent except in chickens: 9.8%. Q/D resistance was among the highest: 5–9% for livestock hosts; 26% in poultry. Striking differences among countries were absent for Ent.
Conclusion: This pan-EU survey, with uniform methodology, shows that antimicrobial resistance among enteric commensal bacteria at slaughter was variable. For Ec, prevalence of antimicrobial resistance varied for older antimicrobials and between countries but resistance to newer medically important antimicrobials was absent or very low. With respect to Ent, antimicrobial resistance rates varied for quinupristin/dalfopristin, but resistance was absent or very low for other antimicrobials including linezolid and vancomycin.

O28 Demography and antibacterial susceptibility of community-acquired respiratory tract infection pathogens in Year 6 vs Year 5 of the PROTEKT surveillance programme

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Objectives: Patterns of antibacterial resistance among community-acquired respiratory tract infection (CARTI) pathogens vary among countries. PROTEKT is a global surveillance study monitoring antibacterial resistance among CARTI pathogens. We report here the results of the sixth year of the PROTEKT study (Y6: 2004–2005) and changes compared to Y5 (2003–2004).
Methods: Clinical isolates of *Streptococcus pneumoniae* (SPN) and *Haemophilus influenzae* (HI) from respiratory samples were submitted from 93 centres from 28 countries. MICs and susceptibilities were determined according to CLSI guidelines. Genotyping was performed to define macrolide resistance mechanisms. Frequencies were compared for Y5-Y6 common sites using χ^2 or Fisher's exact tests as appropriate with $\alpha = 0.05$.
Results: In Y6, a total of 5182 SPN and 1609 HI were collected. SPN/HI distribution by site were: sputum & BAL (55%/72%), blood (19%/2%), nasopharynx (10%/13%), ear (10%/7%), sinus (6%/6%). The distribution of specimens by age groups were: ⩽2 y 12%, 3–14 y 14%, 15–64 y 44%, and >64 y 30%. SPN penicillin and erythromycin resistance (PR and ER) prevalences were 19% and 35% respectively [20% erm(B), 9% mef(A), 5% erm(B)+mef(A), and 1% ribosomal mutation]. SPN with both PR and ER was >25% in Far East, South Africa and France and >15% in Hungary, Poland, US and Australia. Significant increases in PR were seen in Poland and China. Falls in PR were seen in Italy, Spain and Japan. Erythromycin resistance was stable in all countries except Germany and Venezuela (decrease). SPN with multiple drug resistance (>2 antibiotic classes) increased in Poland and decreased in Russia. Prevalence of isolates resistant to at least 5 antibiotic classes increased in China. Beta-lactamase (BL) production in HI was 14% overall, while 2.9% of HI were BL negative and ampicillin resistant (BLNAR). BL production frequencies were stable in all countries except South Africa (increase). Telithromycin showed a sustained activity against both SPN (99.6% susceptible, MIC50/90 0.015/0.25 mg/L) and HI (99.3% susceptible, MIC50/90 1/2 mg/L).
Conclusions: Resistance to several first-line antibacterials is a continuing problem worldwide. The last 2 years of PROTEKT indicate changing patterns of resistance in several countries. Telithromycin exhibited significant in vitro activity against the principal CARTI pathogens, including strains resistant to other agents.

O29 Community-acquired respiratory tract infections in Europe caused by *S. pneumoniae*, *H. influenzae* and *Moraxella catarrhalis*: report from 10 years of monitoring by the SENTRY Program

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

Objectives: To determine the antimicrobial susceptibility (S) patterns for *S. pneumoniae* (SPN), *H. influenzae* (HI) and *M. catarrhalis* (MCAT) when tested by reference CLSI methods using samples collected from 1997–2006 in 13 European (EUR) nations. Trends in S and the occurrences of well defined resistance (R) mechanisms were assessed.
Methods: A collection of community-acquired respiratory tract infections (CA-RTI) pathogens (6,753 SPN; 6,280 HI; and 1,908 MCAT for all years) were annually forwarded to a central reference laboratory for S test processing and confirmation of organism identity. All antimicrobials were tested by CLSI methods, results interpreted by M100-S16 (2006) and quality control rigidly applied to assure accuracy. Analysis of trends used mean S rates by nation for the initial and last 3 years sampled.

Thirty sites and drugs were monitored each year for one-decade. Beta-lactamase (BL) was measured with the nitrocefin test.
Results: SPN strains in EUR were very S to amoxicillin ± clavulanate (A/C; 96.9%), cefepime (FEP; 97.1%), ceftriaxone (CRO; 97.8%), respiratory fluoroquinolones (FQ; 99.0%), rifampicin (RF; 99.2%), tigecycline (98.9%) and vancomycin (100%). Penicillin (PEN)-R (29.9% non-S) and erythromycin (ERY)-R (28.5% non-S) continues to evolve, differing among nations (range, 3.3% [Germany] to 60.6% [Israel] for PEN; range, 4.1% [Sweden] to 52.9% [France] for ERY). Trends toward greater PEN- and ERY-R were noted in 7 and 12 countries, respectively. FQ-R (0.9% overall) indicated by levofloxacin MIC at ⩾4 mg/L was >1% in Belgium, Italy (7.8%; clonal), Spain, UK, Israel and Ireland. MCAT had a uniform BL-production rate (95%, range 92–100%) across EUR and macrolides (MIC90, ⩽0.25 mg/L), tetracyclines (⩽2 mg/L), FQs (⩽0.06 mg/L) and enzyme stable β-lactams remained very potent. BL(+) rates in HI ranged from 3.6% (Germany) to 23.9–30.8% (France, Israel). BLNAR HI strains (Table) were found in 5 nations (range, 0.5–1.7%) with an overall average of only 0.3%. A/C-R was rare (0.4%) and noted in 3 nations; highest (5.5%) in Spain. HI-S rates of >90% were documented for A/C, azithromycin, CRO, FQs, RF, chloramphenicol, oral cephems and tetracyclines.

Key R patterns among CA-RTI pathogens (SENTRY Program, 1997–2006)

Country	R rates (%)[a]				
	PNSP[a]	ENSP[a]	BLP-HI	BLNAR	BLP-MCAT
Belgium	14	29	16	1.7	97
France	48	53	31	0.8	95
Germany	3	19	4	0	92
Greece	60	52	12	0	97
Ireland	33	21	13	0	93
Israel	61	27	24	0	100
Italy	12	48	4	1.2	96
Poland	29	27	8	0	97
Spain	41	38	17	1.2	96
Sweden	6	4	12	0	96
Switzerland	23	22	12	0	95
Turkey	46	25	5	0.5	100
UK	3	7	16	0	93
All EUR	30	28	16	0.3	95

[a]Average of last 3 years of participation. PNSP = PEN-non-S SPN, ENSP = ERY-non-S SPN; BLP = β-lactamase production, BLNAR = β-lactamase-negative AMP-R.

Conclusions: The SENTRY Program has consistently monitored EUR CA-RTI pathogens and observed relatively stable R patterns among HI and MCAT but variable rates among nations. SPN R-rates for PEN, macrolides and to a lesser extent FQs continues to evolve at varying velocities among the 13 countries sampled from 1997–2006. These results corroborate data reported by EARRS for 2004.

O30 Carriage of quinolone-resistant *Escherichia coli* among healthy Israeli Arab children attending daycare centres in northern Israel

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Objectives: To determine the rate, characteristics and risk factors of intestinal carriage of nalidixic acid (NA) resistant *E. coli* (*E. coli* NAR) in healthy children who have never received quinolones as antibiotic treatment.
Methods: Two consecutive surveys, 10 months apart, were carried out to determine the prevalence of *E. coli* NAR in a cohort of healthy children aged 3–5 years from 9 Day Care Centers (DCC) in 3 Arab villages of northern Israel. Parents were interviewed on demographic and socioeconomic characteristics and medical history of the children and their families. Stool samples obtained from the cohort of children and from a sub-sample of siblings and mothers were inoculated onto MacConkey agar plates, containing NA (32μg/mL). The NA Minimal Inhibitory Concentration (MIC) was determined by the broth microdilution technique. The *E. coli* NAR strains were examined for ciprofloxacin resistance. Selected strains were evaluated for efflux pumps activity and for point mutations in the gyrA and parC genes. Uni- and multivariable analyses were used to identify risk factors of *E. coli* NAR carriage.
Results: We found that 17.2% (34/198) of the children carried *E. coli* NAR in the first survey and 42.3 (85/208) in the second. Among the children examined in both surveys (n = 147), 9.4% harboured *E. coli* NAR in the first survey but became negative in the second while 38.5% children found negative in the first survey carried *E. coli* NAR in the second. *E. coli* NAR were isolated in both screenings among 11 (9.4%) children. 21% and 34% of the *E. coli* NAR strains were also resistant to ciprofloxacin in the first and second surveys, respectively. Persistent resistance to NA was associated with an increase in MIC, number of mutations in the gyrA and parC genes and presence of efflux pumps. Acquisition of resistance to NA was significantly higher in two of the DCCs located in one of the 3 villages. The carriage of *E. coli* NAR was not associated with the child's age or gender, use of antibiotics, or carriage of *E. coli* NAR among mothers and siblings.
Conclusions: The lack of evidence for intra-familiar spread of *E. coli* NAR and the significant acquisition of *E. coli* NAR in 2 specific DCCs suggest that other means of transmission such as the food or waterborne routs may explain the high carriage rate of *E. coli* NAR. Persistent carriage of *E. coli* NAR is of concern in view of the association with an increase in both phenotypic and genetic markers of resistance to quinolones.

O31 Antimicrobial resistance of *Neisseria gonorrhoeae* in the Russian Federation in 2006

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Objectives: Antimicrobial resistance in *Neisseria gonorrhoeae* (Ng) remains an important issue affecting treatment recommendations. The present study was designed to ascertain the current status of antimicrobial susceptibility patterns of Ng in different regions of Russia.
Methods: Isolates of Ng were obtained from patients with acute gonorrhoea who visited sexual transmitted disease clinics in 36 regions of Russia from March through October 2006 in a frame of the Russian National Program on Control and Prevention of sexually transmitted infections. The susceptibility to ciprofloxacin, penicillin, ceftriaxone, spectinomycin and tetracycline, was determined by the agar dilution method according to CLSI. Ng ATCC 49226 was used for quality control. Beta-lactamase production was detected by nitrocefin disks (Cefinase; BBL Microbiology Systems).
Results: Among 463 isolates included in the study 73.4% demonstrated resistance to tetracycline, and 73.8% to penicillin; β-lactamase production was detected in 2.8% of isolates; all isolates were susceptible to ceftriaxone. Prevalence of resistance to ciprofloxacin varied in different geographical regions from 45.2% (Volga region) to 100% (Ural region); in total 45.3% of isolates demonstrated high level of resistance, and 5.1% an intermediate level. Amino acid substitutions in GyrA (S91F, D95G), and ParC (S87R) were the main mechanisms of resistance to ciprofloxacin. A high level of resistance to spectinomycin was detected in South region: 19.2%; in the Central, East, North-West, Siberia and Volga regions the rates of resistance varied from 2.9% to 8.6%.
Conclusions: Guidelines for antimicrobial treatment of gonorrhoea were proposed as a result of the present study. In the majority of regions of Russia treatment options are limited to ceftriaxone, and in some regions to ceftriaxone and spectinomycin.

O32 Increasing prevalence of glycopeptide hetero-resistant *Staphylococcus aureus* from the Detroit Metropolitan Area over a 20-year period (1986–2006)

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Background: Glycopeptide hetero-resistance has been associated with clinical failures to vancomycin (VAN) treatment. Recent previous prevalence studies have reported rates as low as 0.7% and as high as 27%. We evaluated the prevalence of hGISA in methicillin-resistant *S. aureus* (MRSA) isolates collected from the Detroit metropolitan area from 1986–2006.
Methods: 1,351 MRSA clinical isolates were screened for hetero-resistance against VAN and teicoplanin (TEIC) using a modified Etest method with a 48 h incubation period and a 2 McFarland inoculum density as described by Walsh et al. (J. Clin. Microbiol. 2001; 39: 2439–2444). Isolates that demonstrated heteroresistance to either vancomycin, teicoplanin or both were considered hGISA. To confirm accuracy of the modified Etest method in detecting hGISA, a subpopulation analysis (PAP) with Mu3 as a control strain were performed on all hGISA strains determined by the Etest method. PAP was performed with an inoculum of 10^9 CFU/mL on brain heart infusion agar plates containing 0, 0.5, 1, 1.5, 2, 3, 4, and 8 mg/L VAN and TEIC. Colonies were counted and plotted against VAN and TEIC concentrations which was then used to calculate an area under the curve (AUC). The criteria used for detection of hGISAs were AUC ratios of $\geqslant$0.9.
Results: We report an increasing prevalence rate of hGISAs from 4% between the years 1986–1993 to 25.1% between 2003–2006. The accuracy of the modified Etest method with PAP was found to be 83.3%. Adjusting for the accuracy of the modified Etest method yielded prevalence rates of ranged from 3.3% to 21% for hGISA between the years 1986–1993, 1994–2002, and 2003–2006, which still demonstrates a significant increase.
Conclusions: The observed prevalence rates of hGISAs represented a substantial increase in the isolates collected over a 20-year period. Based on the inability of current clinical screening methods to detect hGISA, these findings may have serious implications on treatment outcomes with glycopeptides.

O33 Activity of linezolid against a worldwide collection of uncommonly isolated Gram-positive organisms (3,251 strains)

R. Jones, J. Ross, M. Stilwell (North Liberty, US)

Objectives: To assess the worldwide spectrum of linezolid when tested by CLSI reference methods (M7-A7, 2006) against species of Gram-positive cocci that are rare in occurrence (<1%) and are not within the indicated organisms approved by regulatory agencies (US-FDA or EMEA). Using a large surveillance platform (SENTRY Antimicrobial Surveillance Program, 1997–2006), these organisms were tested to provide knowledge of oxazolidinone potency and emerging resistance (R) rates to ensure potential efficacy when needed for chemotherapy of serious infections.
Methods: Linezolid was tested against 3,251 strains distributed among 9 major bacterial groups: *Aerococcus* spp. (22), *Bacillus* spp. (202), Corynebacteria (342), various enterococci (6 species; 378), *Listeria monocytogenes* (137), *Micrococcus luteus* (29), *Rothia mucilaginosus* (18), beta-haemolytic (BST; 3 serotypes, 865) and viridans group streptococci (VGS; 12 spp.; 1,258). All strains were tested by CLSI broth microdilution methods with appropriate supplements; all quality control results were within published CLSI limits (M100-S16, 2006). Linezolid-R strains (MIC, $\geqslant$8 mg/L) were processed by PCR for target site mutations.
Results: Generally, linezolid was very active against all 32 species examined with 4 strains (0.12%) having a MIC at $\geqslant$4 mg/L (*E. avium* [1], *E. casseliflavus* [2], *E. gallinarum* [1]) tested and only 1 R (*S. oralis*; MIC at >8 mg/L) isolate having a proven G2576T mutation. Streptococci (2,123) overall had a MIC90 of 1 mg/L (range, 1–2 mg/L), and the 378 rarely cultured enterococci had a MIC90 of 2 mg/L (range, 2 mg/L). *Corynebacterium* spp. (342) were most susceptible (S) to linezolid (MIC50, 0.25 mg/L) while enterococci and *Listeria* were 4- to 8-fold less S (MIC50, 2 mg/L). Evaluations of R trends in those species failed to identify any MIC creep over the seven years monitored (data not shown).

Table. Linezolid potency against uncommonly isolated Gram-positive organisms (3,251).

Organism (no. tested)	Cumulative % inhibited at MIC (mg/L)					MIC (mg/L)	
	0.25	0.5	1	2	4	50%	90%
Aerococcus spp. (92)	4.5	31.8	63.6	100.0	–	1	2
Bacillus spp. (142)	4.9	31.7	97.2	100.0	-	1	1
B. cereus (60)	3.3	30.0	98.3	100.0	-	1	1
Corynebacterium spp. (238)	68.2	92.4	99.6	100.0	0.25	0.5	
C. amycolatum (11)	100.0	-	-	-	-	0.25	0.25
C. jeikium (59)	67,8	98.3	100.0	-	-	0.25	0.5
C. pseudodiphtherificum (11)	36.4	90.9	100.0	-	-	0.5	0.5
C. striatum (25)	88.0	100.0	-	-	-	0.25	0.5
Enterococcus avium (116)	0.0	4.3	52.6	99.1	100.0	1	2
E. casseliflavus (65)	0.0	0.0	23.1	96.9	100.0	2	2
E. durans (49)	2.0	8.2	32.7	100.0	-	2	2
E. gallinarum (119)	0.0	1.7	30.3	99.2	100.0	2	2
E. hirae (16)	0.0	0.0	37.5	100.0	-	2	2
E. raffinosus (13)	0.0	0.0	46.2	100.0	-	2	2
Listeria monocytogenes (137)	0.0	0.7	29.2	100.0	-	2	2
Micrococcus luteus (29)	3.4	75.9	100.0	-	-	0.5	1
Rothia mucilaginosus (18)	16.7	83.3	94.4	100.0	-	0.5	1
Streptococci (2,123)	4.5	39.5	96.9	99.9	99.9	1	1

Conclusions: Linezolid, the first oxazolidinone to be used in clinical practice, has maintained excellent activity against these rare Gram-positive species as well as frequently cultured and indicated species *S. aureus*, coagulase-negative staphylococci and enterococci (*E. faecium*, *E. faecalis*). All but 5/1 isolates were inhibited by $\leqslant$2/$\leqslant$4 mg/L (>99.8% S) of linezolid if CLSI breakpoints were applied to the testing of these species. Continued R surveillance should be considered for linezolid especially for these rarely isolated Gram-positive species as this agent is more widely used.

Community-acquired methicillin-resistant *Staphylococcus aureus*

S41 Global epidemiology of CA-MRSA

J. Etienne, A. Tristan, M. Bes, H. Meugnier, G. Lina, M-E. Reverdy, F. Vandenesch (Lyon, FR)

Objective: Community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) were described at the beginning of this decade and since 2003 an impressive worldwide spread of Panton Valentine leukocidin (PVL)-positive CA-MRSA clones have been observed. The objective is to characterise the PVL-positive clones spreading presently all over the world.
Methods: A collection of 469 isolates of PVL-positive CA-MRSA isolates collected from around the world between 1999 and 2006 was characterised by the French National Reference Center for Staphylococci.
Results: This study shows that:

i. The continent-specific clones of PVL-positive CA-MRSA described in 2003 have now spread to other continents. Schematically in 2003, ST80 was detected in Europe, ST8 and ST1 in the USA, and ST30 in Oceania. In 2006, intercontinental exchanges of several clones were observed: the ST8 clone (USA300) from the USA towards Europe; the ST1 clone (USA400) from the USA towards Europe and Asia; the ST59 clone (USA1000) from the USA towards Asia; the ST80 clone from Europe towards Asia.

ii. On a given continent, PVL-positive CA-MRSA have spread from country to country. For instance, in Europe, PVL-positive CA-MRSA of ST80 were recently detected in Slovenia, Romania and Croatia.
iii. New PVL-positive CA-MRSA clones are emerging on different genetic backgrounds. While most of the clones described in 2003 had an agr3 background, the newly described clones are agr1 or agr2. Clone ST22 (agr1) has been found in Europe only and clone ST377 (agr1 with a type V SCCmec) was reported simultaneously in Europe and Australia.
iv. PVL-positive CA-MRSA, which were initially susceptible to most antistaphylococcal antibiotics, have acquired new antibiotic resistance determinants, to gentamicin and ofloxacin for instance.
v. The prevalence of PVL-positive CA-MRSA varies considerably from one continent to another (high for instance in the USA (~50%), Greece and Algeria, low in most European countries).
vi. PVL-positive CA-MRSA are gradually causing an increasing number of hospital-acquired infections in countries where their prevalence is high.

Conclusion: To counter this emerging global threat to public health, systematic surveillance of both hospital and community isolates is required, together with measures designed to limit their spread.

S42 The impact of community-acquired MRSA in low-prevalence countries

R. Skov (Copenhagen, DK)

In the Nordic countries and the Netherlands, nosocomial MRSA has successfully been contained for more than 25 years keeping the prevalence of MRSA at <1% in these countries. This has been accomplished by enforcing preventive contact isolation of all hospitalised patients suspected or confirmed as MRSA positive. This has been facilitated by the fact that in these countries the predominant risk factor for patients for MRSA acquisition was hospitalisation abroad, making these at-risk patients relatively easy to identify. Additionally, secondary cases seldom arose after hospital discharge.

The recent change in epidemiology where transmission of MRSA increasingly occurs outside hospitals has changed the situation, and an increasing number of MRSA has been reported in all the above mentioned countries.

Since the mid-1990s the number of new cases of MRSA in Denmark has increased by 10-fold. The majority of these MRSA has been identified in primary healthcare (community onset) and, in approximately 33% of the cases, the patient had had no connection with healthcare institutions for at least one year prior to the diagnosis. These cases have therefore been considered as community acquired (CA-MRSA).

CA-MRSA infections have especially been reported from children and younger adults. Skin and soft tissue infections dominate and represent approximately 90% of the cases. Approximately 30% of CA-MRSA cases have been reported in persons with another ethnicity than Danish. This could indicate that there is a continued influx of MRSA from high prevalence areas into Denmark. Strains belonging to the ST80-IV complex have been the most frequent cause of CA-MRSA in Denmark, however, an increasing diversification has recently been observed including almost all strains known as associated with CA-MRSA.

During the same time period the number of MRSA diagnosed in hospitals also increased. This has happened despite continued use of contact isolation of MRSA positive patients during. Hospitalisation of MRSA carriers not identified on admission due to lack of risk factors is probably one important factor responsible for the increased number of nosocomial MRSA in Denmark.

An increase in the prevalence of MRSA outside hospitals including in otherwise healthy persons, is thus of great concern as this not only gives rise to CA-MRSA infections but also inevitably will increase the risk of nosocomial MRSA infec-tions and outbreaks.

In order to keep Denmark as a low prevalence country we believe that MRSA should be contained and eradicated not only in hospitalised patients but also when diagnosed in the community. This has lead to new national guidelines for preven-tion and eradication of MRSA including making both carriage and infection with MRSA mandatory reportable.

S43 How, when and whom to screen

K. Christiansen (Perth, AU)

The first question to ask when considering any screening programme for the identification of CA-MRSA carriage is why? The importance of epidemiological data cannot be underestimated. Data on prevalence can inform empirical therapy requirements or help in the design of intervention strategies. Prevalence can be established for the population in general, or, specific subgroups such as children, military personnel, indigenous or disadvantaged populations can be targeted. Community intervention measures similar to the 'search and destroy' techniques used in hospitals would require large-scale screening. A narrower approach concentrating on high-risk groups to enable programmes such as education on hygiene to reduce transmission would allow a more targeted screening programme. A second more pragmatic area for screening is on admission or during hospitalisation. In some areas in the world there is a blurring of the boundaries of CA-MRSA and HA-MRSA. These organisms have now been reported as a cause of hospital acquired bacteraemia, surgical and prosthetic joint infection and to colonise neonates and adult patients in hospitals. Targeted screening of high-risk patients or all patients on entry into high-risk units are strategies to prevent CA-MRSA from becoming endemic in hospitals. Screening to control recognized outbreaks entails identification of the reservoir to enable appropriate isolation, cohorting and decolonisation measures. The value of screening healthcare workers for carriage of CA-MRSA has yet to be established.

How to screen is the next question. Which site(s) give the best yield? Which laboratory method should be used? The ideal test would have high negative and positive predictive values, be rapid, inexpensive, applicable to the routine laboratory, and have high through put capabilities. Currently used tests are traditional microbiological culture and identification methods, use of selective media including various types of CHROMagar and molecular techniques using real time PCR. The requirement for broth amplification increases the sensitivity for some of these methods but also increases the time to identification. There are many different CA-MRSA clones in the world, in Western Australia alone there are over 40 different multi locus sequence (MLST)/SCCmec CA-MRSA clones. Any molecular test must therefore have the ability to identify all clones in diverse regions of the world.

The last questions are when and whom to screen. The answer is very much dependent on the purpose of the screening. The range can be from large-scale periodic population screening for epidemiological purposes to continual hospital unit, high-risk patient screening.

There are no established guidelines or recommendations for screening for CA-MRSA. Many questions have yet to be answered particularly, whether control of transmission in the community is possible. By looking at what evidence is available we can move a step closer to control of this very significant community pathogen.

S44 Optimisation of control strategies and treatment

S. Cosgrove (Baltimore, US)

Rates of infections caused by CA-MRSA continue to increase, causing over 50% of skin and soft tissue infections seen in the emergency department in a recent report. Strategies for control of CA-MRSA in patients and their families include use of infection control techniques in the household as well as decolonisation with mupirocin, chlorhexidine, and antibiotics in some cases. Given that CA-MRSA has been now been implicated in healthcare-associated infections, efforts to reduce the spread of this organism within the healthcare environment are also important and include appropriate hand-hygiene, isolation, and environmental decontamination. Incision and debridement remains critical in the management of most CA-MRSA infections. The use of antibiotics is indicated in many CA-MRSA infections and therapeutic

options include older agents, such as clindamycin, trimethoprim-sulfamethoxazole, tetracyclines, and rifampin, and newer agents such as linezolid. In addition, new agents are in development.

Pathogenesis and epidemiology of invasive pneumococcal infections

S45 Recent discoveries in the pathogenesis of pneumococcal pneumonia

D.H. Dockrell (Sheffield, UK)

Pneumococcal pneumonia is a pathogen of global significance and represents a leading cause of infection-related mortality at all ages. Recent developments in the field of pneumococcal pathogenesis reflect significant developments in both genomics and proteomics. The breadth of microbial genetic diversity is increasingly being recognized. Our understanding of the importance of traditional virulence factors such as polysaccharide capsule, pneumolysin and generation of hydrogen peroxide is evolving while important roles for factors such as pneumococcal pili are emerging. Genetic screens are identifying increasing roles for previously poorly characterised proteins involved in metabolism and transport of key molecules. The importance of the physical state of the bacteria in different tissue compartments is also becoming evident. An improved range of models of various aspects of infection ranging from colonisation and sub-clinical infection to fulminant pneumonia and invasive disease are allowing assessment of both microbial and host factors central to disease pathogenesis. The range of receptors involved in the innate response to pneumococci in the respiratory tract is starting to be appreciated and includes Toll-like receptors and receptors primarily involved in the phagocytosis of bacteria. The interaction between soluble factors and the resident phagocytes of the lung is being clarified and the complexity of cytokine networks involved in the innate host response is being elucidated. Important roles for T-lymphocytes, Natural Killer cells and dendritic cells in the response to pneumococci are being identified. Furthermore our understanding of the regulation of the inflammatory response and of the significant role of apoptosis in the regulation of the inflammatory response has lead to important insights into how cell survival and outcome of infection are closely linked. These observations are enabling a more scientific interpretation of many of the central clinical features of pneumococcal pneumonia and may encourage novel approaches to therapy to improve disease responses.

S46 Invasive disease potential of pneumococcal clones carried by children

H. de Lencastre, R. Sá-Leão, M. Ramirez, J. Melo-Cristino (Oeiras, Lisbon, PT)

Streptococcus pneumoniae (the pneumococcus) continues to be a leading cause of morbidity and mortality worldwide particularly among young children, the elderly and the immunocompromised of all ages. Infection is preceded by colonisation of the nasopharynx, which is the ecological niche of pneumococci. In most individuals colonisation is asymptomatic and does not evolve to disease. The carrier state is more frequent in young children and may reach over 70% in some populations such as those attending day-care centres. Indeed, this latter group has been found to be a major reservoir of pneumococci playing a key role on the amplification and transmission of the bacteria to other individuals. Of interest, several drug-resistant clones internationally disseminated have been found to be frequently carried by this population. In recent years, there has been some debate on the relative contribution of serotype and genetic background to the invasive disease potential of pneumococcal strains. We will present a study that addresses this question. The study was conducted in Portugal and results from the detailed comparison of two large collections of pneumococcal isolates: a group of over 450 invasive disease isolates and a group of over 750 colonisation isolates collected during the same time period which were characterised in detail by antibiogram, serotype, PFGE macro-restriction profiles, and multilocus sequence typing. The invasive disease potential of serotypes and clones will be discussed.

S47 Epidemiology of invasive pneumococcal infections

A. Brueggemann (Oxford, UK)

Pneumococci cause a spectrum of diseases in humans, from reasonably mild diseases like sinusitis and conjunctivitis to potentially life-threatening diseases like meningitis and bacteraemia. The current conjugate vaccines are ideally aimed at protecting against all pneumococcal disease, but have been very successful at preventing invasive pneumococcal disease. Understanding invasive disease epidemiology, both preceding vaccine implementation and after vaccine introduction, is crucial to the design and development of future vaccines. Several recent studies have shown that pneumococcal serotypes differ in their invasive disease potential, and this has particular relevance for the selection of serotypes to include in future conjugate vaccines. It is also essential to understand the serotype-specific changes that have occurred subsequent to conjugate vaccine implementation in the USA, as a model for what might occur post-vaccine implementation in other countries.

S48 Recent discoveries in prevention of pneumococcal disease

K. Klugman (Atlanta, US)

The recent history of pneumococcal disease prevention is dominated by the development in the 1990's of conjugate pneumococcal vaccine for administration to infants, and its implementation starting in 2000 in the USA. The vaccine as currently formulated affords protection against invasive pneumococcal disease (IPD) due to the 7 most common serotypes causing IPD among children less than 2 years of age prior to vaccine introduction in the USA. This formulation is not optimal for many developing countries where serotypes such as 1 and 5 are important. Global, second generation vaccines therefore include at least 10 serotypes. The importance of prevention of pneumococcal disease extends beyond IPD to pneumonia, meningitis, otitis media, prevention of antibiotic-resistant infections, and even prevention of mortality in young infants. Each of these presents specific challenges. The vaccine has been shown to prevent between 25% and 37% of all cause X-ray confirmed pneumonias and this implies a level of protection against pneumococcal pneumonia due to vaccine serotypes in excess of 70%. This reduction in pneumonia has translated into a reduction in all-cause mortality due to the vaccine of 16% in rural Africa. The major preventable burden of disease in Africa may be among HIV infected children where the vaccine has also been shown to reduce pneumonia burden. An innovative aspect of the vaccine is the fact that it may reduce the pneumococcal super infections that follow viral respiratory infections, such as influenza, and conjugate vaccination of children may thus be a useful adjunct to pandemic influenza planning preparedness. The interruption of carriage of vaccine serotypes has been shown to reduce IPD in adults in the USA due to herd immunity so the population benefit in an influenza pandemic may extend beyond protection just of immunised children. Herd immunity has also recently been shown to protect young un-immunised infants from IPD. Conjugate vaccine has had little impact on all cause otitis media in randomised trials, but post-marketing studies in the USA suggest that healthcare utilisation for otitis episodes has reduced significantly post vaccine introduction suggesting a major role for herd immunity and allowing physicians to feel more comfortable in a "wait and see" attitude to otitis once the prospects of significant complications due to the pneumococcus are likely to be rare. The conjugation of pneumococcal polysaccharides to *Haemophilus influenzae* protein D has allowed the development of a more efficacious vaccine against otitis media. There has been a dramatic reduction in antibiotic resistance among vaccine serotypes causing IPD in the USA, in both children and adults, but resistance, driven by continuing high levels of antibiotic use, is now selecting resistance in non-vaccine

serotypes. In particular, multiresistant global clones of serotype 19A are now dominant in the USA and a 13-valent vaccine formulation including serotype 19A is under development. The implementation of immunisation programmes including conjugate pneumococcal vaccine to infants in European countries affords the opportunity to monitor the impact on all of these health outcomes in both children and adults.

Infective endocarditis – time for change?

S50 Treatment options for infective endocarditis: new drugs for bad bugs?

R. Corey (Durham, US)

In the USA infections due to both hospital and community-acquired methicillin resistant *Staphylococcus aureus* (MRSA) are increasing rapidly in both frequency and severity. As a result new treatment options are badly needed.
This presentation will first address the role of traditional therapy focusing on the gradual development of increasing resistance to vancomycin among MRSA. Next it will focus on alternative oral therapies such as clindamycin, cotrimoxazole and minocycline and their role in the treatment of community-acquired MRSA, an organism that has displaced "traditional" MRSA in many venues. The efficacy of the newly approved antibiotics linezolid, daptomycin, and tigecycline will be addressed through the presentation of the results of large phase 3 clinical trials in complicated skin and skin structure infections, hospital-acquired pneumonia, and bacteraemia. Discussion will focus on the efficacy of these agents in the MRSA subgroups. Finally, the efficacy of antibiotics in development will be presented again focusing on the MRSA subgroups from phase 2 and 3 studies. These antibiotics will include dalbavancin, telavancin, oritavancin, iclaprim, ceftobiprole and ceftaroline.
As the epidemiology of staphylococcal infections changes new therapeutic agents are becoming available to clinicians. Therapeutic utility will depend on understanding the unique characteristics and efficacy of the new agents in clinical trials in order to clearly understand their role in the new therapeutic paradigms.

New tricks for old drugs

S53 Recent and emerging uses of long-acting tetracyclines

H.M. Lode (Berlin, DE)

Antimicrobial resistance is a serious problem with increasing strains of bacteria becoming resistant to many or all available antibiotics. Tetracyclines were already introduced 50 years ago but have undergone a considerable resistance development in nosocomial and community-acquired pathogens. In June 2005, a minocycline derivative, the new glycylcycline antimicrobial tygecycline, was approved by the FDA for treatment of complicated intra-abdominal and complicated skin and skin-structure infections. Tigecycline, a bacteriostatic agent, binds to the 30S ribosome unit, blocks entry of amino-acyl tRNA molecules, and prevents protein synthesis; it has a broad antibacterial spectrum with activity against Gram-positive and Gram-negative pathogens, including multidrug-resistant organisms and anaerobes. The modification of the basic tetracycline molecule has overcome the two resistance mechanisms seen with tetracyclines (efflux, ribosome protection). Tigecycline exhibits linear pharmacokinetics, has a long half-life (24–48 hours), is highly protein bound (71–89%), and has a large volume of distribution (7–9 L/kg).
Tigecycline is not extensively metabolised; the primary route of elimination is biliary excretion (59%) and 33% is excreted unchanged in urine. The recommended dosing regimen is an initial dose of 100 mg, followed by 50 mg every 12 hours. Time above MIC and AUC/MIC ratio are the most important pharmacodynamic parameters.
In two double-blind studies the safety and efficacy of tigecycline versus aztreonam plus vancomycin were compared in hospitalised adults with complicated skin and skin-structure infections. Over 1000 patients were randomised and the results of the pooled analysis determined that tigecycline monotherapy was as effective and statistically non-inferior to aztreonam plus vancomycin.
Two double-blind studies including more than 1000 patients analysed safety and efficacy of tigecycline versus imipenem/cilastatin in adults with complicated intra-abdominal infections. Results of the pooled analysis determined that tigecycline was efficacious and statistically non-inferior to imipenem.
Nausea (20–30%) and vomiting (15–20%) are the most common adverse effects observed in clinical trials. They usually occur within the first two days of therapy, are more common in women (48%) than in men (24%), and may also be age related.
Tigecycline is an important addition to the antimicrobial armentarium. It has a broad spectrum of activity and its ability to evade numerous mechanisms of resistance makes it a promising solution to the treatment of multi-drug resistant organisms. This is very helpful in selected patients in the ICU.

S54 Parenteral colistin: finally a useful drug?

F. Jacobs (Brussels, BE)

The increasing resistance of Gram-negative bacteria and particularly amongst *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, raises a major therapeutic problem. The lack of new effective agents in the near future has revived interest in the re-use of some molecules, abandoned because of their toxicities. Colistin (colistimethate sodium) is a polymyxin antibiotic available in parenteral formulation and used for intravenous, aerosolised and intrathecal/intraventricular administration. The bactericidal activity of colistin is due to a detergent effect on the cell membrane and therefore not dependent upon bacterial metabolic activity. Because this drug was developed during the 1950s, only little pharmacokinetic and pharmacodynamic information are available and the optimal daily dose for severely ill patients is still unknown.
Colistin, used both intravenously and by inhalation, was used until last decade mainly in cystic fibrosis patients. Recently, colistin has been used in non cystic fibrosis patients as a salvage therapy for the treatment of infections with Gram-negative bacteria resistant to all other antibiotics. Clinical reports on the efficacy of intravenous colistin in these patients are not randomised studies and in most of them, colistin was frequently associated with other antimicrobial agents with a lack of a control group. A good outcome occurred in most infections (52–75%) but with the poorest results observed in pneumonia (25%).
In vitro studies have shown synergistic effect between colistin and rifampin but no clinical study has been designed to confirm this synergy. Nephrotoxicity and neurotoxicity are the most common toxicities of colistin. However recent studies demonstrated a reduced toxicity as compared with previous studies, even if a higher dosage is used. This low renal toxicity rate was observed in both critically ill patients and patients treated with prolonged courses of colistin. There are also recent clinical reports on the use of colistin in continuous intravenous infusion to minimise potential toxicity. This reduction of toxicity without impairment of efficacy (concentration-dependent killing in in-vitro time-kill studies) should be confirmed by further studies. Neurotoxicity has also been more frequently reported in case of renal dysfunction.
Development of resistance to colistin is a major problem, especially in *A. baumannii*. This highlights the urgent need to preserve this molecule by the best appropriate dose regimen and by an optimal synergistic combination with other antibiotics.

S55 Facts and myths about fosfomycin

F. Gudiol (Barcelona, ES)

Fosfomycin is a phosphonic broad spectrum antibiotic discovered in Spain in 1969, licensed in many countries since then, but used only sporadically in the clinical setting. In fact, It is not mentioned specifically in the current editions of the most prestigious textbooks of internal

medicine or infectious diseases. The emergence of infections caused by bacteria resistant to almost all antimicrobial agents may have renewed the interest for addressing the contributions, limitations and future clinical indications of this drug.
Fosfomycin exhibits a rapid bactericidal activity against a large number of aerobic Gram positive and Gram negative bacterial species by inhibiting the initial step of cell wall syntesis. Some important multiresistant pathogens such as penicillin-resistant pneumococci, methicillin-resistant staphylococci, vancomycin-resistant enterococci and ESBL-producing enterobacteriaceae are usually susceptible to the drug. To date, no cross-resistance with other antibiotics has been reported. Favourable pharmacokinetic properties include a low molecular weight, a negligible protein binding, a large volume of distribution in human tissues and a good penetration into the inflamed CSF. The disodium salt of fosfomycin can be administered intravenously in high doses due to its very low toxicity, achieving plasmatic peak levels that are several times above the MIC of susceptible microorganisms (breakpoint 64 g/mL). An oral salt of fosfomycin and tromethamine with enhanced bioavailability is also available since 1990.
The major drawback of fosfomycin is the frequent development of resistance during therapy. This phenomenon, which has been demonstrated in "in vitro" and "in vivo" studies, precludes its parenteral use as a single agent in the clinical practice. As a counterbalance, fosfomycin can act synergistically with other antibiotics, especially with those that inhibit later points in cell-wall synthesis. Such synergism has been shown repeatedly against different strains of *Staphylcoccus aureus*, CNS, *Streptococcus pneumoniae* and *Pseudomonas aeruginosa*. Moreover, in experimental animal models of MRSA endocarditis and cefalosporin-resistant pneumococcal meningitis, fosfomycin and β-lactam (or vancomycin) combinations have proved to be superior than monotherapies, preventing the emergence of drug-resistant populations as well. However, the reported clinical experience with these combinations is very scarce and, therefore, there are not evidence-based recommendations supporting its use.
On the other hand, various clinical trials have indicated that fosfomycin trometamol, as a sole antibiotic, is effective and safe for the treatment of uncomplicated UTI, providing high and long lasting urinary fosfomycin levels, which help to prevent the emergence of resistant strains. Because of the increasing prevalence of CTX-M type ESBL-producing *E. coli* in the community, the oral administration of a single dose of 3 gr. of fosfomycin trometamol may be considered nowadays as a first line therapy for uncomplicated UTI in young women. However, the possible emergence of fosfomycin-resistant mutants among ESBL-producing enterobacteriaceae should be taken into account.
In conclusion, after 40 years of its discovery, only the oral form of fosfomycin – tromethamine – has attained a solid position in the antimicrobial guidelines. Combinations of iv fosfomycin with other antibiotics may be considered in selected cases, but whether these combinations yield improved outcomes in specific infections due to multirresistant pathogens remains to be determined. Logistic reasons and financial limitations will make difficult the realisation of appropriate prospective studies to answer this issue in the near future.

S56 Chloramphenicol: Goodbye Hello

W. Graninger (Vienna, AT)

Chloramphenicol is still used extensively in non-industrialised countries for the treatment of severe infections like pneumonia and typhoid fever because it is cheap and effective.
Chloramphenicol proved to be an effective alternative for the treatment of pneumococcal and meningococcal disease and also in patients with meningitis caused by *Haemophilus influenzae*. Chloramphenicol's loss of favour began in the 1960s, when it was shown to have two distinct toxic effects on hematopoiesis: a frequently observed, dose-dependent anaemia, reversible on cessation of therapy, and an irreversible, 'idiosyncratic' aplastic anaemia, which had an incidence of approximately 1 case per 30,000 courses of therapy, a high case fatality rate, and no correlation with the or duration of treatment. The use of chloramphenicol has then become restricted to life-threatening infections for which there are no acceptable alternative treatments like brain abscesses.
Analogues of chloramphenicol have been developed that lack the aromatic NO_2 group that is thought to cause irreversible marrow aplasia. Although the clinical efficacy of such compounds has not been evaluated, they are effective in vitro against most bacteria, even those that are resistant to chloramphenicol. The data provided in support of a hypothesis that these molecules needed a nitro group in order to cause the blood dyscrasias were insufficient in themselves. Florfenicol has followed chloramphenicol in veterinary medicine. The potential for florfenicol to cause blood dyscrasias, such as aplastic anaemia, in humans was discussed in relation to the chemically related chemicals chloramphenicol and thiamphenicol. Human epidemiological data could not be used to demonstrate the safety of florfenicol as florfenicol has never been used in human medicine. Nevertheless, because there is as yet no way to monitor the potential for irreversible bone marrow toxicity, it is unlikely that such compounds will become available for the treatment of chloramphenicol-resistant infections in general – and meningococcal disease in particular.
The future of chloramphenicol is not entirely certain. On one hand, it is a well-studied, long-standing treatment for fighting a number of different infections; it is also very inexpensive and consequently has very widespread use. It would, however, be a very good thing if we could find a new drug or group of drugs that produced similar results in fighting infection but did not have as many serious side effects. It would take a long time before any drug could ever replace chloramphenicol, because of both its broad use and its very low cost.

Tropical medicine: from basic science to field work

O57 Evaluation of malaria status and immune response to *Plasmodium falciparum* MSP-2

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Objectives of study: MSP-2 is a highly polymorphic 45–53 kDa merozoite surface antigen and very immunogenic malaria antigen, which is considered as a promising vaccine candidate. The 3 S and 5 S end regions of the gene are highly conserved, whereas a large central region is variable. Many studies have suggested a protective role for specific IgG antibodies against variable regions of MSP-2. This study was designed to analyse the reactivity of human sera from people living in a malaria-endemic area of The Gambia (village of Keneba) against different domains of MSP-2. The association of haemoglobin and parasitaemia as two indicators of clinical malaria with acquired immunity were analysed to elucidate the pattern of protective immunity.
Materials and Methods: The current study was designed and carried out in Liverpool School of Tropical Medicine. 179 human sera were randomly selected from McGregor's Keneba Sera Collection (1966–1980). Different domains of MSP-2 were synthesized using GST gene fusion system and crude schizont extract was prepared from in vitro culture of *Plasmodium falciparum*. Total IgG and IgG subclass responses were measured by ELISA after a checkerboard study was performed for each antigen to standardise the concentration of both antigens and antibody.
Results: Most sera predominantly recognized the immunodominant regions of the molecule. Increasing the age was negatively correlated with parasitaemia and positively with IgG antibody responses and haemoglobin levels indicating that total IgG responses to domains 2, 3 and crude schizont extract coincidental to a decrease in parasitaemia density and frequency. IgG3 response was the main IgG in those who had no parasitaemia at the final time point. IgG2 and IgG3 were increased amongst individuals with no parasitaemia and with higher levels of haemoglobin at higher ages that had exposure to parasite over a long period of time.
Conclusion: IgG3 was the main antibody, mainly against domain 3 of MSP-2, that was associated with increase in haemoglobin levels and

decrease in parasitaemia suggesting that domain 3 is preferred over other domains and crude schizont extract in presenting a clearer picture of immunity against malaria. These results could be an evidence of protective role of these antibodies against malaria disease and, therefore, domains 3 can be considered as reliable vaccine candidate antigens.

O58 Presence of dengue virus genome in the bone marrow of asymptomatic adults in a dengue-hyperendemic country: implication for complicated dengue pathogenesis

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Objectives: Our country is hyperendemic for dengue virus infection. Serosurveillance indicates that almost all native adults have been infected, mostly asymptomatically. A long-held mechanism explaining clinical severity involves sequential infections by different serotypes. Even though some of its peer flaviviruses are known to reside persistently within the host and contribute to host illnesses, dengue virus has not been shown to behave in a similar fashion. As dengue is a haematotropic virus, we sought to find evidence of its persistence in the bone marrow of previously-infected persons.
Methods: We studied patients clinically suspected or known of haematologic malignancies and indicated to have diagnostic bone marrow initial or follow-up studies. A fraction of cellular marrow was employed for RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR) by dengue-specific primers. Serologic assessment by haemagglutunation inhibition test (HI) for dengue and chikungunya viruses and by enzyme-linked immunosorbent assay (ELISA) for dengue was performed on the study of bone marrow study and, in some cases, 14–60 days later, to minimise a chance of including patients with recent dengue infection. Serologic interpretation was made according to standard criteria. Demographic data of all patients were analysed, especially for the history of recent febrile illnesses which could be due to dengue infection.
Results: Of 83 enrolled patients, dengue genome was detected in cellular marrow of 6 cases (table). All these 6 cases were in the stage of remission of their haematologic diseases. They had had no prior history of recent febrile illnesses and HI and ELISA results of single or paired sera from these patients were compatible with either remote (#1–5) or remote/no (#6) infection by flaviviviruses. As for the rest, one may have had fairly recent dengue infection and one had rising titers for chikungunya virus.

Patients with dengue genome in the bone marrow

Patient # & diagnosis	age & gender	1st, 2nd HI of serotype with highest titers	ELISA: 1st, 2nd IgM & 1st, 2nd IgG
1. B cell lymphoma	44y F	1:160, NA	2, NA & 16, NA
2. Multiple myeloma	48y M	1:40, NA	24, NA & 1, NA
3. Multiple myeloma	52y M	1:40, NA	4, NA & 3, NA
4. CML	56y M	1:40, 1:80	0, 1 & 4, 6
5. B cell lyrnphoma	38y F	1:40, 1:40	0, 0 & 2, 2
6. CML>	54y F	<1:20, <1:20	0, 0 & 10, 5

Abbreviations: CML, chronic myelogenous leukaemia; F, female; M, male; NA, data not available.

Conclusions: Sequential infections by different serotypes seem to be a key in severe dengue pathogenesis. Its peer flaviviruses, however, have been shown both in vitro and in vivo to do so. The persistent first-serotype dengue genome, defective or complete, could possibly confer a biological influence when co-infected with a second serotype later on in their life. Our understanding of dengue pathogenesis is far from perfect, and this finding may open up a door to a new arena of dengue research.

O59 Dynamics of *Plasmodium falciparum* alleles in children with normal haemoglobin and with sickle cell trait in western Uganda

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This is the first study to characterise *Plasmodium falciparum* population in relation to haemoglobin type in western Uganda.
General objective: To investigate the composition of *P. falciparum* in children aged 3 months to 15 years in Western Uganda and characterise the genotype of *P. falciparum* with the longest duration of carriage in sickle cell trait. Specific objectives: To study the allelic variants of *P. falciparum* in children aged 3 months to 15 years reporting at Mbarara University Teaching Hospital and Kagando Hospital, in western Uganda; to analyse the duration of carriage of specific parasite genotypes in relation to the type of haemoglobin of the children; to characterise *P. falciparum* harboured by asymptomatic malaria carriers with sickle cell trait; to investigate the effect of turnover of *P. falciparum* genotypes on parasite density.
Methods: Microscopic identification of malaria parasites and estimation of parasitaemia was done using Giemsa-stained thick and thin blood films. Haemoglobin phenotype was determined by electrophoresis of blood samples on cellulose acetate membrane in alkaline buffer. Nested PCR using specific primers for merozoite surface protein (MSP) 1 and 2 allelic families was used to genotype *P. falciparum* in 291 isolates collected from children in two districts Mbarara and Kasese.
Results: Extensive genetic diversity was detected among symptomatic children in Mbarara (20 MSP1; 31 MSP2 alleles) and Kasese (19 MSP1; 30 MSP2 alleles). Multiplicity of infection (MOI) with MSP2, a high polymorphic genetic marker, was higher in Kagando than in Mbarara being 2.1 and 2.7 genotypes/PCR positive-sample, respectively. The difference in MOI in children in the two districts was statistically significant (MSP1 $P < 0.0001$; MSP2 $P = 0.036$). Clear differences in the distribution of individual alleles of FC27, IC and RO33 were apparent in the study areas. A deletion of a 12-amino acid sequence in RO33 160 bp allele yielded RO33 130 bp, which predominated in symptomatic children in Mbarara. About 13% of asymptomatic children in Kisinga, Kasese carried HbAS. MOI was age-dependent and higher in children with HbAS than with HbAA. Haemoglobin type influenced the distribution of FC27-type alleles among asymptomatic children Kagando.
Conclusion: *P. falciparum* polymorphism is extensive in western Uganda, and most of the infections are composed of multi-clonal infections. HbAS increases MOI and is important in development of naturally acquired immunity.

O60 TLR-cascade in malaria: role in antiparasitic clearance

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Objectives: Host innate immunity is important for early parasite control but also contributes to disease pathology. Antigenic variability of the parasite as well as host immune response mechanisms have been analysed in numerous studies. Yet, little is known about the initial recognition of parasite antigens by host immune receptors in blood stage malaria.
Methods: In a murine malaria model, the role of the TLR cascade for parasite clearance and for initiating innate and cell mediated immunity was analysed. Mice deficient for TLR2/4, TLR4, TLR9, and MyD88 as well as controls were infected with non-lethal Plasmodium yoelii. Parasitaemia and survival were assessed. In addition, on day 6 post infectionem cytokine levels were measured in serum and in cultured spleen cells after 48 h stimulation with anti CD3. To assess T cell stimulation, respective activation markers were assessed by flow cytometry.
Results: In vivo, MyD88 –/– mice showed significant higher parasite loads and a mortality of 38% compared to 0% in controls. However, time

needed to completely eliminate parasite from blood in surviving mice did not differ between groups. Parasitaemia in TLR2/4 –/– and TLR9 –/– mice and in controls was similar. MyD88 –/– mice had significantly lower levels of IL-12 and IFN-γ. T cell proliferation as well as T-cell activation was not altered.
Conclusion: MyD88 plays an important role in eliminating blood stage parasites. This is reflected by reduced levels of co-stimulatory as well as pro-inflammatory cytokines.

O61 TLR2, but not TLR4 mediates *Onchocerca volvulus* induced corneal inflammation

K. Daehnel, E. Pearlman (Cleveland, US)

Objectives: This study was designed to identify the role of TLR2 and TLR4 in the development of *Onchocerca volvulus* induced keratitis.
Methods: Bone-marrow derived dendritic cell cultures were obtained from C57Bl/6, TLR2 –/–, TLR4 –/– and TLR2/4 –/– mice and stimulated in vitro with *O. volvulus* extract. Dendritic cell activation was measured by IL-6 and RANTES production and by CD40 expression by flow cytometry.
C57Bl/6 and TLR2 –/– mice were then immunised with *O. volvulus* extract and soluble antigen was injected into the corneal stroma. Neutrophil and eosinophil migration into the corneal stroma was determined by immunohistochemistry, and filaria-specific IL-5 and IFN-γ production by splenocytes was measured by ELISA.
Results: Incubation of dendritic cells with filarial extracts induced production of IL-6 and RANTES and up-regulation of CD40. In contrast, there was no elevation of any of these factors in dendritic cells obtained from TLR2 –/– or TLR2/4 –/– mice, but dendritic cells from TLR4 –/– mice responded similar to C57Bl/6 dendritic cells.
Immunisation of C57Bl/6 and TLR2 –/– mice caused increased filaria-specific IL-5 production by splenocytes. In contrast, IFN-γ production was only increased in splenocyte cultures from C57Bl/6 mice, but not in cultures from TLR2 –/– mice. In the C57Bl/6 corneas, injection of filarial antigens induced neutrophil and eosinophil infiltration. In TLR2 –/– mice, neutrophil migration into the corneal stroma was completely abrogated, whereas eosinophil migration remained unaffected.
Conclusion: We conclude that TLR2 plays an important role in early recognition of filarial antigens, the induction of filaria-specific T cells and neutrophil migration to the corneal stroma.
Ongoing studies are examining the role of IL-6 and IFN-γ in the development of adaptive immune responses and corneal inflammation.

O62 Prevalence of *Hymenolepis nana* and other intestinal parasites as mixed infection among children in Ilam, and impact of single-dose praziquantel against *H. nana*

A. Khosravi, A. Dalimi Asl, A. Kaikhavandi (Ilam, IR)

Objective of study: This study was designed to evaluate the prevalence of intestinal parasitic infections in primary and secondary School children in relation to some individual and social risk factors such as the parent's job, the water supply source, raw vegetable consumption, area of living, gender, etc., in Ilam and assess the impact of two different doses of Praziquantel against *H. nana* infection.
Methods: The mixed infection of *Hymenolepis nana* and other intestinal parasites were studied in a 3 years prospective study using 1140 stool samples that were randomly collected from 5 regions of the city, the North, the East, the West, the South and the City Centre. Samples were tested using microscopy, direct examination, and then Formalin Ether diagnose tests were carried out to compare the results. Three groups each of 30 infected individuals were selected randomly and they were given Praziquantel 15 mg/kg, 20 mg/kg and Placebo respectively.
Results: The overall prevalence of *H. nana* was 13.4% with no significant difference for males and females. The parent's jobs, age of children and source of water supply had no significant correlation with the prevalence of infection while a strong correlation was found with raw vegetable consumption. 46.96 percent of all children were shown to have parasitic infection with the highest prevalence reported for Trichuriasis and Giardiasis, 26% and 25.30% respectively. Amongst 34 percent of individuals who had *H. nana* infection Giardia Lamblia were also diagnosed as a mixed infection; 3% of individuals who had *H. nana* infection were diagnosed with Taenia Saginata infection too. Abdominal disorders, lack of appetite, dizziness, vomiting and diarrhoea were the main reported symptoms. The cure rate of Praziquantel against *Hymenolepis nana* infection was about 100% using both doses of drug.
Discussion: There is a high rate of parasitic infection, some with mixed infection, in children of primary and secondary schools in Iran with higher prevalence for those living in poor parts of country like Ilam. *H. nana* is a worldwide parasitic infection distributed amongst children affecting their growth and also their quality of study. The high prevalence and also the mixed infection reported in this study suggested that the Health Services should work more effectively and promote their impact on the knowledge, attitude and practice of children. Praziquantel cured the infection using single dose appropriately which is in agreement with other studies.

O63 Chagas' disease: a growing problem in Spain

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Background: Chagas' disease is a parasitic infection caused by *Trypanosoma cruzi*. It is endemic in Central and South America and, during the last few years, a large number of migrants from these areas have settle in Spain. Therefore the diagnosis of Chagas'disease has become a common event in our clinical practice.
Methods: We have reviewed all cases of Chagas' disease during the last 2 years. Specific serology (ELISA) was the diagnostic procedure. Epidemiological, clinical, cardiac ultrasound, serological and therapeutic aspects were analysed.
Results: During 2004 and 2005 we studied 7 patients with Chagas' disease. 3 men and 4 women with a mean age of 38.8 y (24–51 y). 85% were from rural areas of Bolivia (Cochabamba and Valle del Potosí) and one patient came from the North of Argentina. All patients had positive antibodies against *T. cruzi* performed in their countries. The main clinical complaints were: dizziness (1p), dyspnea (2p), palpitations (1p) or asymptomatic screening (3p). 2 cases had positive xenodiagnosis. All cases but 2 were asymptomatic, having normal ECG and cardiac ultrasound. 2 women had arrhythmia: one atrial fibrillation and ventricular tachycardia requiring a DAI and the other had ventricular dysfunction and constipation. All cases but one previously treated received therapy with benznidazole for 30–60 days with good tolerance. A pregnant woman did not receive treatment.
Conclusions: The new entry of immigrants to Spain, coming from rural endemic areas of Chagas' disease of Bolivia and other Southamerican countries should make us suspect this entity in young people with cardiac or gastro-intestinal disturbances.

O64 Survival of dengue virus in the urine of acutely-infected patients – implications for pathogenesis and for a possible unrecognized mode of transmission for an arthropod-borne virus

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Objectives: Dengue virus infection is considered the world's most important arthropod-borne disease. We have earlier reported a pilot study that dengue, like some other viruses, can be detected by RT-PCR in urine from a number of patients, even in an early postfebrile period. Here we wish to report a follow-up study with more cases and with our success in isolating the virus from early and late urine specimens of acutely-infected patients.
Methods: Hospitalised patients suspected of dengue infection were enrolled. Diagnosis was confirmed by standard criteria of enzyme-linked immunosorbent assay (ELISA) and/or haemagglutination inhibition test (HI). Patients with negative dengue serologies served as negative

controls. Dengue-specific PCR, with primers targeting conserved sequences in the 3′-untranslated region, was performed in urine specimens with a strict and properly-evaluated protocol. Pairs of febrile and postfebrile urine specimens from 1 adult and 3 paediatric dengue cases with positive dengue-specific PCR in urine, together with those of 1 adult and 2 paediatric negative controls, were processed and then employed for Aedes aegypti intrathoracic inoculation. To minimise urinary toxicity to mosquitoes, 3 forms of processed urine were used: diluted urine pellets, diluted urine supernatants filtered though 0.2-μm membrane, and diluted urine supernatants mixed with antimicrobials. Each form of each specimen was inoculated into 20 mosquitoes. Surviving mosquitoes were dissected 14 days after inoculation. Dengue-specific PCR was performed on extracts from the body parts of all injected mosquitoes.

Results: Of 237 dengue patients enrolled, the virus was detected in 122/189 paediatric and 20/48 adult urine specimens by PCR (64.55% and 41.67% respectively). Live dengue viruses were detected in all febrile and postfebrile urine specimens of dengue cases but in none of negative controls (Table). The virus was isolated as late as 14 days after defervescence. Filtered urine supernatants served as the best specimen type, with the least urinary toxic effects to mosquitoes.

Specimens	# cases with virus isolated/total # cases		
	urine pellets	urine supernatants	
		Antimicrobials added	Microfiltration (0.2 μ)
Dengue			
febrile urine	1/4 (25%)	3/4 (75%)	4/4 (100%)
postfebrile urine	0/4 (0%)	3/4 (75%)	4/4 (100%)
Nondengue			
febrile urine	0/3 (0%)	0/3 (0%)	0/3 (0%)
postfebrile urine	0/3 (0%)	0/3 (0%)	0/3 (0%)

Conclusion: Dengue virus is not only excreted in urine in at least half of the patients, but it is also live and culturable. These findings have implications for dengue pathogenesis and for public health. Urine is implicated as a potential mode of transmission for certain viruses. Whether the arthropod-borne dengue will be added to the list is subject to further investigation.

O65 Spatiotemporal variations of malaria incidence and protective efficacy of intermittent preventive antimalarial treatment of infants

J. May, R. Kobbe, B. Kreuels, S. Adjei, O. Adjei on behalf of the Agona IPTi Trial Team

Background: Intermittent preventive antimalarial treatment of infants (IPTi) is considered a promising malaria control strategy. Among the factors that influence the extent of protection provided by IPTi are malaria endemicity, seasonality, drug resistance patterns and the IPTi application schedule. Studies modeling the effect of malaria incidences on IPTi are scarce. The aim of this study was to describe how far protective efficacy of IPTi depends on the incidence rate of clinical malaria.

Methods: One-thousand seventy infants were enrolled in a registered controlled trial on the efficacy of sulfadoxine-pyrimethamine based IPTi in the Ashanti Region, Ghana. In an ecological analysis, malaria incidence rates in the first year following IPTi were stratified by the village of residence and month of birth of participants and the spatiotemporal variation of the malaria incidence on the protective efficacy of IPTi was analysed.

Findings: The rate of malaria attacks during the first year of follow-up was highly dependent on the month of birth and on the village of residence of the children. Protective efficacy of the first IPTi administration (IPTi-1) correlated with malaria incidences in children living in a particular village or born in a particular month (r2 0.48, $p < 0.04$ and r2 0.63, $p < 0.003$, respectively). A corresponding trend was seen after the second (IPTi-2) and third (IPTi-3) drug administration.

Interpretation: Correlations between IPTi efficacy and malaria incidences may have implications on IPTi implementation strategies and, most likely, on that of other malaria control measures.

Influenza A: update on epidemiology, virology and pandemic preparedness planning

S83 The role of mathematical modelling in pandemic preparedness

S. Cauchemez, N.M. Ferguson (London, UK)

I will discuss the role mathematical modelling in pandemic planning and response. Recent research examining whether antiviral prophylaxis and social distance measures could be used to contain a nascent pandemic at its point of origin will then be reviewed. Containment is potentially feasible, but requires rapid detection of the initial transmissible case cluster and a rapid and organised response to each new case. These may be demanding criteria for much of SE Asia. If containment fails, slowing spread becomes a policy priority and in that context I will discuss the potential impact of restrictions on international travel. To conclude, I will discuss pandemic mitigation strategies which make best use of limited vaccine and antiviral supplies.

Infection in cancer patients (jointly arranged with the International Immunocompromised Host Society)

S84 Update on the epidemiology of infections in cancer patients

G. Maschmeyer (Potsdam, DE)

Infections are among the most frequent complications occurring in cancer patients undergoing antineoplastic chemo- or immunotherapy. Invasive fungal infections today represent the main causes of fatal outcome. Early diagnosis of probable or proven invasive aspergillosis is therefore one of the most important objectives of supportive care in patients with profound and prolonged neutropenia. With the advent of more effective and well-tolerated antifungals active against aspergillosis, the incidence of other mould infections such as zygomycosis is on the rise.

The use of highly aggressive chemotherapy induces severe mucosal damage in many cancer patients. Apart from neutropaenic enterocolitis, a broad spectrum of infections associated with impairment of mucosal barriers may be clinically important, e.g., streptococcal bacteraemia, septic enterococcal infection, or candidaemia. The widely spread use of multi-lumen central venous catheters causes a considerable number of bloodstream infections caused by coagulase-negative staphylococci, *S. aureus*, Gram-negative bacilli, or *Candida* spp. Primary removal of foreign material may be important for the successful management of these catheter-related infections.

Allogeneic hematopoietic stem cell transplantation with reduced-intensity conditioning has become more common also for treatment of aggressive haematologic malignancies in elderly patients as well as in patients with severe co-morbidity, who formerly have not been taken into consideration for myeloablative transplant procedures. Despite a marked reduction of complications related to pancytopenia combined with acute graft-versus-host reaction, the rate of severe and life-threatening fungal infections and cytomegalovirus diseases has turned out to be comparable to conventional allogeneic transplantation. Most importantly, more than half of these infectious complications emerge after more than 90 days post transplant, i.e., in patients already managed on an outpatient basis.

The broad introduction of monoclonal antibodies to CD20 and CD52 and of nucleoside analogs into the treatment of patients with B- or T-cell lymphomas has lead to long-term depletion of B cells, eventually associated with decreasing levels of serum immunoglobulins, and T-cell deficiency lasting for many years. Particularly in patients being treated with these compounds for relapsed or refractory malignancy, a high number of infections caused by pathogenic viruses such as CMV, invasive fungi or *Pneumocystis jiroveci* are observed. Targeted prophylaxis is warranted for selected patient groups, whereas high alertness and early pre-emptive antimicrobial intervention is mandatory in the majority of these patients.

Tuberculosis: fast forward to new approaches

S88 VNTR typing as the next gold standard in the molecular epidemiology of tuberculosis

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In the last decade, IS6110 restriction fragment length polymorphism (RFLP) typing has gained recognition as the gold standard in the molecular epidemiology of tuberculosis. The application of RFLP typing has provided new insights in, e.g., the natural history of tuberculosis infection and (inter-)national transmission of multi-drug resistant tuberculosis in Europe (to be presented). However, this method is technically demanding and labour intensive, and requires weeks of culturing to obtain sufficient DNA. The complex RFLP patterns are difficult to interpret and exchange.

The new generation genotyping of *Mycobacterium tuberculosis* – based on the detection of the variable number of tandem repeats (VNTRs) of mycobacterial interspersed repetitive units (MIRUs) – is increasingly used to solve these problems. VNTR typing is based on PCR amplification of multiple genomic loci to determine the number of tandem repeats at these sites. This typing is much easier to perform than IS6110-RFLP typing, and is applicable to crude low-concentration DNA extracts. Current studies are even oriented to apply VNTR typing directly to clinical material. Moreover, the results are expressed as numerical codes and are, therefore, easy to compare and exchange.

Recently, in an international collaboration, the resolution, stability and technical applicability of 29 MIRU-VNTR loci was compared. This study comprised the initially explored 12 loci (as applied in the USA) and most of the exploitable loci disclosed in the international context so far. The typing results of 824 *M. tuberculosis* isolates revealed the 24 most optimal loci. The use of 15 selected loci is recommended as the new international gold standard for typing of *M. tuberculosis* complex isolates. A recent study pointed out that VNTR typing on basis of 24 loci is useful to study the phylogeny of the complex.

As experienced after the standardisation of IS6110 RFLP typing in 1993, it is expected that the recent (December 2006; J. Clin. Microbiol.) international consensus on VNTR typing will facilitate the comparison of molecular epidemiological data from various geographic regions, the spread of multi-drug resistance in Europe, the establishment of an international VNTR database and the meta-analysis of worldwide typing results in order to study the population structure and spread of *M. tuberculosis*.

Many institutes in the world have large databases of IS6110 RFLP patterns of *M. tuberculosis* isolates from extended time periods. Because in the epidemiology of tuberculosis transmission and manifestation of the disease can be separated in time by multiple years, the switch to VNTR typing can not be done without any overlap of the application of the two typing methods. During the ECCMID meeting the first re-typing results in The Netherlands and considerations underlying the choice of isolates to be re-typed will be discussed.

S89 Genetic aspects of tuberculosis: persistence and pathogenesis

L.W. Riley (Berkeley, US)

A large proportion of new cases of tuberculosis (TB) in the world arise from a reservoir of people latently infected with *Mycobacterium tuberculosis* (Mtb). In TB endemic areas of the world, the average time to diagnosis a case of TB after the onset of active disease is 6 months. During these 6 months, such a case of TB is likely to infect, on the average, 10 close contacts. If one of these 10 newly infected contacts then develops TB sometime in his life time, he will infect 10 other persons before diagnosis. The cycle then repeats itself. Thus, if a TB control programme is only focused on the treatment of active cases, TB will never come under control. The fundamental question in TB control, therefore, is "can we predict who among the latently infected people will progress to active disease so that such a person can be given preventive treatment?" To answer this question, the mechanism of Mtb persistence and reactivation needs to be better understood. A variety of models for bacterial persistence has been proposed recently. Most genetic studies that provide evidence for the putative role of a gene in persistence are based on demonstrating that an Mtb strain disrupted in such a gene is attenuated in an animal model of TB. However, several recent gene disruption studies have shown that Mtb mutants can also become hypervirulent in an animal model. This suggests that some Mtb genes may actually help to temper the virulence potential of Mtb so that the organism can establish a stable niche in a host without harming the host. We provide evidence that a cluster of genes in an operon called mce1 in Mtb is involved in lipid transport and metabolism that helps to remodel the bacterial cell envelope. The operon appears to respond to a signal induced by the host's granuloma cell turnover, while the host's proinflammatory cells that comprise the granulomas appear to respond to the changing bacterial cell envelope lipid turnover. An Mtb mutant disrupted in the mce1 operon becomes hypervirulent in a mouse model of TB; it is unable to induce well-organised granulomas in mouse lungs, and the mouse dies from an uncontrolled bacterial proliferation. This mutant accumulates free mycolic acids on its surface. On the other hand, an Mtb strain disrupted in the negative regulator of the operon (mce1R) constitutively expresses the mce1 genes in vivo. This mutant is also hypervirulent but for an opposite reason-it causes rapidly progressive, hyper-proinflammatory response. Thus, the mce1 operon appears to serve as a homeostatic regulator of pro-inflammatory response by the host, helping to establish a balanced relationship favourable to both the host and Mtb. Understanding and exploiting this relationship may lead to new ways to predict a subset of latently infected people who progresses to active disease; it may also lead to the development of a therapeutic vaccine to prevent latently infected people from ever developing TB.

S90 Global overview of new anti-TB compounds

N. Doi (Tokyo, JP)

General trend: In response of the serious clinical demands, and the initiative of the Global Alliance for TB drug Development (TB-Alliance), 7 new Anti-TB drug candidates: nitroimidazopyran (PA-824; Chiron), moxifloxacin (MFLX: Bayer), gatifloxacin (GFLX; Kyorin), diarylquinoline (TMC-207; Johnson & Johnson), nitroimidazo-oxazole (OPC-67683; Otsuka), Pyrolle LL-3858 (Lupin) and diamine SQ-109 (Sequella) are in progress in clinical trials phase-I to -III. Majority of the novel candidates listed above lack interactions with iso-enzyme CYP3A4 of P-450, indicating available into the combination therapy with anti-retroviral drugs for the treatment of HIV/TB co-infection cases. Among them, PA-824, TMC-207, SQ-109 and OPC-67683 proved to be effective against (X)MDR-TB; they have been studied to introduce into the first-line drugs in near future.

New candidates developing in Japan: (1) OPC-67683 (Otsuka Pharm) shows a specific potent activity only against *M. tuberculosis*, whereas it is ineffective against clinically important NTM (non-tuberculous mycobacteria) species including *M. avium* and *M. intracellulare*. OPC-67683 is now in progress at clinical trial phase-II. (2) Recently, DC-159a,

a novel respiratory quinolone was synthezised (Daiichi Pharm), showing a potent anti-mycobacterial activity against Quinolone-Resistant-MDR- or XDR-*M. tuberculosis*. DC-159a exhibits the best gEBA (early bactericidal activity: after 3-, 6- and 9-day treatment) h in murine TB model, which was superior to those of MFLX, LVFX, INH and RFP. DC-159a is in progress in preclinical stage.

Toward the next generation chemotherapy: To fight with (X)MDR-TB, Latent-TB as well as HIV/TB, next generation of standard combination chemotherapy is urgently required. Varieties of different classes of novel candidates allow an encouraging scope for the establishment of 3 to 4 months of short-course treatment of TB in near future. Now, new projects are launching to shorten the total treatment duration, based on the systematic PK/PD study and drug-drug interaction study among current-/novel-anti-TB drugs and anti-retroviral-drugs.

S91 The global tuberculosis epidemic: burden, challenges and the international control strategy

M.C. Raviglione (Geneva, CH)

Tuberculosis (TB) is a disease of poverty that impedes development. Its global burden is huge. WHO estimates that, in 2005, 8.8 million new cases occurred world-wide and that 1.6 million patients died of TB. The vast majority of cases and deaths (>90%) were in the developing countries. The highest incidence rates (>300 cases per 100,000 population) were in sub-Saharan Africa, and the highest numbers of cases (about 60% of all) were in Asia. However, TB is everywhere and there is no country in the world that has eliminated it or is near elimination. The HIV epidemic has fuelled TB incidence in many settings, especially in Africa. Multidrug-resistant TB (MDR-TB) is particularly frequent in the former USSR and in China (up to 14% of all new cases), and extensively drug-resistant TB (XDR-TB) has emerged in all continents and caused major concern in South Africa, where it has appeared among persons living with HIV/AIDS. Globally, the curve of TB incidence seems to have flattened, largely the result of the possible peaking of the epidemic in Africa. In other regions, incidence is stable or in decline, thanks to recent control efforts.

Clear targets for TB control exist, as defined within the Millennium Development Goals (MDG) and by the Stop TB Partnership. To reach a stable decline in incidence (MDG 6) and to halve prevalence and mortality by 2015, several challenges must be faced. First of all, DOTS (the 5-element essential strategy promoted by WHO since 1995) needs to be fully implemented everywhere and of high quality, so that diagnosis, treatment and monitoring can be guaranteed in all settings and for all patients, and the operational targets of at least 70% case detection and 85% cure can be achieved. Second, the association of TB and HIV and the emergence of severe drug resistance require specific policies and interventions in most countries. Third, general health systems and services, especially at primary care level, must be robust enough to allow TB control and care practices to be effective; hence, national TB programmes need to engage in the broad discussion on health system strengthening and contribute to it. Fourth, it is clear that in most settings in 2007, many non-state sector practitioners or some public practitioners not linked with national programmes, are delivering sub-standard TB care. Thus, it is necessary to ensure that they are fully engaged in sound care delivery. Fifth, communities are playing an increasingly important role in contributing to their health; however, too few are adequately informed on TB and active in its care and control, thus losing an opportunity to mobilise more resources and increase political commitment at all levels. Finally, research is still badly under-funded and too slow in delivering new tools that could radically improve current practices in diagnosis, treatment and prevention. A renewed effort is necessary if new tools are to be made available quickly.

To face these challenges, WHO has recently launched the new Stop TB Strategy, built around the cost-effective DOTS approach. The Strategy consists of six components: (i) pursue high quality DOTS expansion and enhancement; (ii) address TB/HIV, MDR-TB and other challenges; (iii) contribute to health system strengthening; (iv) engage all care providers; (v) empower people with TB and communities; (vi) enable and promote research. If fully implemented, this new Strategy will allow a proper response to the modern challenges to TB control. The Stop TB Strategy underpins the Global Plan to Stop TB 2006–2015, which defines the financial requirements and the gaps to be filled in order to reach the 2015 goals.

Pathogenesis of bacterial infections

O92 Presence of a pneumococcus-like capsulation locus in viridans group streptococci

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Objective: To study the presence of pneumococcal virulence genes in viridans group streptococci

Methods: 109 viridans group streptococci from respiratory samples of hospitalised patients and *Streptococcus mitis*, *S. pneumoniae*, *S. oralis*, *S. infantis*, *S. peroris*, *S. sanguinis* and *S. parasanguinis* type strains were tested for presence of the genes for capsular polysaccharide synthesis protein A (cpsA), competence stimulating peptide (comC), autolysin (lytA), choline kinase (licA), hyaluronidase (hya), pneumococcal surface adhesin A (psaA), pneumococcal surface protein (pspC) and pneumolysin (ply).

Results: Four strains (termed Sv29, Sv5, Sv26 and Sv30) contained the cpsA fragment. Sv29 contained licA and SV29 plus Sv5 contained comC. API20, PCR identification based on the species-specific D-Ala:D-Ala ligase gene (Garnier, JCM 1997, 35, 2337), whole cell protein profiling, high-resolution genotyping (AFLP) and sequence analysis of the 16S rRNA gene confirmed that Sv5, Sv26, Sv29, and Sv30 clustered in a single group and belonged to the *S. mitis* group. It is not clear whether they belong to a known species or constitute a new species within the *S. mitis* group.

Strain Sv29 was sequenced up- and downstream of cpsA. Approximately 25 kb of DNA (9 kb upstream of the cpsA start and 15 kb downstream) were comprised between an upstream dexB and a downstream aliA gene. 14 genes were found between the cpsA start and the aliA stop, comprising genes identical to the pneumococcal capsulation genes wzg (capsular polysaccharide expression regulator), wzh (Wze phosphotyrosine phosphatase), wzd (Wze phosphorylation), wze (autophosphorylating protein-tyrosine kinase), wchA (undecaprenyl phosphate glucose-phosphotransferase), wzy (oligosaccharide repeat unit polymerase), wzx (flippase) and several genes with putative glycosyl, glycerol and acetyl transferase activity and a putative UDP-galactopyranose mutase. Four genes were upstream between the cpsA start and the dexB start, comprising dexB, aliA-like orf1 and 2 genes and an unidentified gene. Expression of the capsulation locus genes was demonstrated. A Quellung reaction with multivalent antiserum to detect capsular polysaccharides remained negative in Sv29 and in Sv5, 26 and 30.

Conclusion: We report for the first time the presence of a pneumococcal-like capsulation locus in *S. mitis* group streptococci that are not *S. pneumoniae*. These genes may represent a new an unknown source of capsule variation in *S. pneumoniae*

O93 Membrane cofactor protein (CD46) binding to clinical isolates of *Streptococcus pyogenes*: binding to M type 18 strains is independent of Emm or Enn proteins

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Previous studies have shown that Membrane cofactor protein (MCP) CD46 is the cellular receptor of keratinocytes for *S. pyogenes* of the M types M6, M5 and M22. However, data using other M types and cells have cast some doubts on the role of CD46 as the main receptor for *S. pyogenes* in all human cells.

Objective: The aim of the present study was to analyse the binding of CD46 to clinical isolates of *S. pyogenes* of different M types.

Methods: Twenty six *S. pyogenes* clinical isolates from different origins and expressing different M types were included in this study. To investigate the role of the hyaluronic acid capsule, the Emm18 protein and the Enn18 protein, we used a set of mutants derived from the wild type strain 87–282 including the Emm18 protein deficient mutant 282KZ and the capsule deficient mutant TX72. Isogenic mutants TX74 and TX76, derived from TX72, deficient in the Emm18 protein and the Enn18 protein, respectively, were also included in the study.
Binding of CD46 was determined by incubation of the bacteria with purified recombinant soluble human CD46. CD46 binding to each strain was determined by Western blot analysis using policlonal anti-CD46 antibodies. Binding of CD46 to purified recombinant Emm18 protein was determined by ELISA.
Results: Binding assays showed that CD46 binding to *S. pyogenes* was highly variable among the 17 different M types tested, being M type 18 strains among those showing the strongest binding. The binding of purified human CD46 to M type 18 strains was independent of the expression of the hyaluronic acid capsule, since the highly mucoid strain 87–282 bound CD46 as efficiently as the derived capsule deficient mutant TX72.
Surprisingly, the Emm18 protein deficient mutants 282-KZ and TX74 bound CD46. Moreover, CD46 did not bind to purified recombinant Emm18 constructs, suggesting that the Emm18 protein is not involved in the binding of CD46 to the M type 18 strains. To test another protein candidate for CD46 binding to *S. pyogenes* M type 18, we generated the strain TX76. TX76 bound CD46 as efficiently as the strains TX72 and TX74.
Conclusion: Binding of human CD46 to *S. pyogenes* is highly heterogeneous and do not depend on the presence of hyaluronic acid capsule. Despite Emm proteins have been assumed to mediate binding of *S. pyogenes* to CD46, M type18 strains bind CD46 very efficiently through a cell surface protein different from the Emm and Enn proteins.

O94 Recognition of *Staphylococcus aureus* by plasmacytoid dendritic cells is species-specific but not related to alpha-toxin or protein A expression

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Objectives: Plasmacytoid dendritic cells (pDC) represent the major source of type I interferons (IFN-I) in the human body. IFN-I are secreted in response to viral infection and represent early mediators of innate immune defence. Less is known about the role of IFN-I in bacterial infections. Since *S. aureus* has been shown to induce IFN-I we asked whether other staphylococci stimulate human pDC and addressed the molecular mechanism of staphylococcal IFN-I induction.
Methods: pDC were isolated from PBMC of healthy donors by positive selection with anti-BDCA4-coated microbeads. Cells were stimulated with bacteria (1:5) in 96 well plates (0.5×10^6/mL) for 24 hours. IFN-α concentrations were measured in the supernatants by ELISA. Alpha-toxin and protein A gene expression in clinical isolates was measured by PCR. Recombinant alpha-toxin and protein A were used to stimulate pDC. *S. aureus* strains Cowan I and Wood 46 were purchased from DSMZ.
Results:
– All *Staphylococcus aureus* isolates stimulated IFN-α secretion from human pDC.
– All clinical isolates of coagulase-negative staphylococci failed to display significant stimulatory activity.
– Only viable *S. aureus* cells stimulated significant amounts of IFN-α.

Based on these results we assumed that *S. aureus* may stimulate pDC by secreting a *S. aureus*-specific substance:
– Since alpha-toxin has been shown to activate immune cells we stimulated pDC with recombinant alpha-toxin or with clinical *S. aureus* isolates typified as alpha-toxin positive or negative by gene analysis. Alpha-toxin failed to induce IFN-α secretion and *S. aureus* isolates stimulated pDC independent of alpha-toxin gene expression.
– Recombinant protein A (SpA) failed to promote IFN-α synthesis from pDC. Moreover, no differences between Wood 46 strain (SpA-deficient) and Cowan I strain (high producer of SpA) were observed. Induction of IFN-α by clinical *S. aureus* isolates was not dependent on SpA gene expression.

Conclusions: Stimulation of human pDC by *S. aureus* is species-specific since coagulase-negative staphylococci fail to induce significant IFN-I responses. Since viability of *S. aureus* is required for pDC stimulation we assumed that *S. aureus* activates pDC via secretion of *S. aureus*-specific molecules. Two major pathogenic factors, SpA and alpha-toxin, failed to stimulate IFN-α synthesis. We conclude that other molecules specific for *S. aureus* are recognized by pDC, thus triggering IFN-I defence against pathogenic staphylococci.

O95 A teicoplanin-resistant mutant of methicillin-resistant *Staphylococcus aureus* exhibits increased uptake and improved survival in non-professional phagocytes

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Background: Significant pleiotropic changes in genes coding for virulence and autolysis have been observed in glycopeptide-intermediate strains of *S. aureus*. We previously reported higher expression of fibronectin-binding proteins (FnBPs), known to promote endocytic uptake of *S. aureus* by epithelial and endothelial cell lines, by a teicoplanin-resistant (TeiR) isogenic derivative (strain 14–4) compared to its teicoplanin-susceptible (TeiS) MRSA parent (strain MRGR3) or its TeiS revertant (strain 14–4rev). In contrast, TeiR strain 14–4 was shown to display lower levels of mRNAs coding for alpha-toxin and its global regulator agr, which are potentially involved in lysis of eukaryotic cells. This study analysed the potential impact of these molecular changes on bacterial invasion and survival.
Methods: Quantitative uptake of TeiR and TeiS strains was evaluated after 2 h of incubation at 37ºC with human embryonic kidney 293 cells at a 10:1 ratio, by a lysostaphin protection assay, and expressed as percentage of control strain *S. aureus* Cowan I uptake. Differential intracellular survival of all *S. aureus* strains from 2 to 24 h was evaluated by CFU counts of Triton X-100-lysed 293 cells, following lysostaphin inactivation.
Results: Endocytic uptake of TeiR strain 14–4 was equivalent to that of Cowan I but 4-fold higher than that of TeiS strains MRGR3 and 14–4rev. Even more spectacular differences were recorded for the endocytic survival of strain 14–4, which showed an 11-fold increase in CFU counts at 24 h, as opposed to Cowan I and the TeiS strains MRGR3 and 14–4rev, whose intracellular CFU counts showed marginal (<2-fold) changes from 2 to 24 h. Infected 293 cells showed no evidence of lysis at 24 h, as assessed by the Trypan blue dye exclusion test.
Conclusions: The increased endocytic uptake of TeiR strain 14–4 compared to its TeiS counterparts may be a consequence of the higher expression of FnBPs by the TeiR strain. The improved intracellular survival and proliferation of TeiR strain 14–4 is an interesting in vitro observation, whose cellular and molecular basis has yet to be explained and would deserve further investigations. Intracellular location might confer a significant fitness benefit to glycopeptide-intermediate isolates of MRSA, not only in experimental in vitro models but also in relevant in vivo situations.

O96 *Staphylococcus aureus* in cystic fibrosis patients: Agr-alleles, resistance and virulence determinants

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Objectives: *Staphylococcus aureus* can be a respiratory pathogen in cystic fibrosis (CF) and its pathogenicity is related to a combination of virulence and antibiotic-resistance genes. Little is known about the agr-group in CF-isolates.
This study investigated the antibiotic-resistance, the distribution of 16 virulence determinants in 98 *S. aureus* strains (single or serial isolates-same strains removed), subdivided in agr-classes, isolated during a period of 18 months from the sputum of 60 CF patients, receiving various courses of antibiotic-therapy.

Methods: The following were performed: agr-typing by ScaI-RFLP method; investigation of virulence genes by multiplex-PCR; antibiotic-resistance by the disk diffusion method according to the CLSI guidelines.
Results: MRSA was isolated in 8% of cases. All staphylococci showed the following resistance profile: 37% for ERY, 21% for GM, 5% for DA, 14% for CIP, 11% for LVX, 12% for RD, 6% for TE and 3% for C. Among the strains, agrII was the most prevalent (35%) while agrI-III-IV were isolated approximately in 21% of cases. Within the agr-groups the distribution of virulence determinants revealed a common conserved-core background (sarA-rnaIII-spa-icaA-hla-hlg), whereas accessory-genes were always represented, in different combinations, and co-possessed significantly. Capsule type-5 was prevalent in agrI while type-8 was prevalent in agrII/III. agrI was associated with the presence of many virulence-genes such as cytotoxins and proteases (sea-lukE- splB) and in one agrI isolate lukS/F-PVL was found; agrII also possessed an adhesin sdrE; agrIII was associated with adhesins and enterotoxin (fnbA-sea-cna), the exfoliatinA was never detected; agrIV possessed the cytotoxin lukE, adhesin cna and exfoliatinA, eta. Protease gene (splB) was always found associated with cytotoxin lukE in agrI-II-III and, mostly, sdrE adhesin was associated with these. A frequent inverse-correlation was found between cna and lukE-splB in all agr-groups and a close correlation was observed between cap8 and cna in agrIII-IV.
Conclusion: Our data demonstrated for the first time an increase of MRSA in our centre; furthermore, CF-persistent infections were not associated with a distinct agr-specificity group or a diffused antibiotic-resistance of the strains, but rather *S. aureus* isolates have a complex distribution and combination of virulence determinants which contribute to *S. aureus* pathogenicity in CF patients.

O97 Increased proliferation of dentate granule cells in human bacterial meningitis

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Objectives: Proliferation and differentiation of neural progenitor cells in the dentate gyrus is increased after infection in animal models of pneumococcal meningitis.
Methods: To evaluate neurogenesis in patients with acute infection of the central nervous system, brain sections of 18 patients dying from bacterial meningitis and 8 patients dying from non-neurological diseases were investigated by immunohistochemistry.
Results: In the dentate gyrus of the hippocampal formation, the density of Proliferating Cell Nuclear Antigen (PCNA)-expressing cells was higher in patients with bacterial meningitis compared to the control group ($p = 0.02$). Furthermore, the number of cells expressing the immature neuronal marker protein TUC-4 was increased in brain sections of persons dying from bacterial meningitis compared to control cases ($p = 0.004$). In the subventricular zone, no difference of cells expressing TUC-4 was observed between both groups ($p = 0.39$). Immature neurons expressing TUC-4 had no morphological features of apoptotic cell death and no evidence of DNA fragmentation.
Conclusion: The increased proliferation of neural progenitors suggests that endogenous mechanisms may limit consequences of neuronal destruction after meningitis. Stimulation of neurogenesis might help to improve therapy of acute inflammatory diseases of the brain.

O98 Continuous stimulation of cerebral Toll-like receptor 9 by intraventricular CpG-DNA causes chronic inflammation, ependymal damage and deficits of memory function

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Objectives: Toll-like receptors (TLR) are important factors of innate immunity. TLR9 is a ligand for bacterial DNA or synthetic CpG-DNA oligonucleotides and stimulates the immune response by activation of the MyD88 complex. In our experiments, the effect of chronic TLR9 activation by CpG-DNA in the central nervous system (CNS) was investigated.
Methods: C57Bl6-mice ($n = 21$) were trained to find a hidden underwater platform within less than 90 s (18 trials over 3 days). Swim tracks and the latency to escape from the water were recorded by a video camera. Thereafter, mice received CpG-DNA (0.001 mg/d) or the same volume of sterile saline over 28 days into the right ventricle after stereotactic implantation of a catheter connected to a subcutaneous osmotic pump. Water maze testing was peformed weekly.
Results: After initiation of treatment, the latency to reach the hidden platform in the water maze was sigificantly longer in mice receiving CpG-DNA than in control animals ($p = 0.006$ at day 28 after initiation of treatment). Histology showed profound inflammation with invasion and activation of T- and B-cells and axonal damage. Furthermore, ependymal damage and loss was seen in CpG-treated animals. Additional experiments with TLR9-deficient mice showed no major inflammation after CpG-treatment over 28 days.
Conclusion: Chronic activation of TLR9 in the CNS causes inflammation, ependymal loss and memory deficits.

O99 Increased fibronectin levels in the cerebrospinal fluid of patients suffering from bacterial meningitis aggravate toll-like receptor induced inflammation in primary mouse microglial cells

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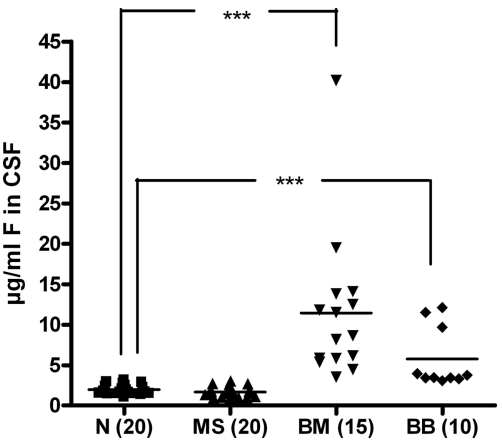
Objectives: Fibronectin, an extracellular matrix (ECM) protein, is discussed to be a potent stimulator of the innate immune system through Toll-like-receptor 4. Toll-like receptors (TLR) play a key role in the recognition of products from virtually all classes of pathogenic organisms. In central nervous system (CNS) infections several ECM proteins and bacterial compounds are elevated in the cerebrospinal fluid (CSF). For this reason, we hypothetised that endogenous and pathogen-derived molecules may jointly stimulate the innate immune system and increase the neuronal damage in bacterial meningitis.
Methods: Levels of fibronectin were measured in the CSF of patients suffering from bacterial meningitis, multiple sclerosis and with an elevated CSF protein due to other causes and compared to healthy controls. In primary cultures of mouse microglial cells the interaction of endogenous and exogenous stimulators of the innate immunity was examined after application of defined Toll-like receptor (TLR) agonists [lipopolysaccharide (LPS) (TLR4), tripalmytoyl-cysteinyl-seryl-(lysyl)3-lysine (Pam3Cys) (TLR2) and single-stranded unmethylated cytosine-guanosine (CpG) oligodesoxynucleotide (TLR9)] alone and in combination with fibronectin. Supernatants of stimulated glial cultures and unstimulated controls were analysed for nitric oxide (NO) and tumour necrosis factor-alpha (TNF-α).



Fibronectin in CSF

Results: Fibronectin was elevated in the CSF of patients suffering from bacterial meningitis and with an elevated CSF protein due to other causes, but not in patients with multiple sclerosis and in healthy

control patients. Co-administration of fibronectin in a concentration which occurs in bacterial meningitis [10 μg/mL] with CpG, LPS or Pam3Cys to primary cultured microglial cells led to an additive release of NO and TNF-α.
Conclusion: The inflammatory reaction to the TLR agonists CpG, LPS and Pam3Cys in primary cultured microglial cells is enhanced by fibronectin in concentrations occurring in CSF during CNS infections. Exogenous–endogenous Co-activation leads to stronger microglial stimulation and may be in part responsible for neuronal damage occurring in these diseases.

O100 *Streptococcus agalactiae* invasion of human brain microvascular endothelial cells is promoted by the laminin-binding protein

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Objectives: *Streptococcus agalactiae* (*S. agalactiae*) can cause severe pneumonia, sepsis and meningitis in neonates and remains one of the most prevalent causes of invasive neonatal infections. During the course of infection, *S. agalactiae* colonises and invades a number of host compartments, thereby interacting with different host tissues. In this study, we investigated the role of *S. agalactiae* laminin-binding protein (Lmb) on adherence to and invasion of human brain microvascular endothelial cells (HBMEC).
Methods: In standard adherence and invasion assays we evaluated the capacity of scpB-lmb and lmb mutants of *S. agalactiae* to adhere to and invade into HBMEC and performed inhibition studies using wild-type *S. agalactiae* in combination with recombinant Lmb or polyclonal Lmb-antibodies. Furthermore interleukin (IL)-8 release was detected by ELISA.
Results: Deletion of the scpB-lmb region, coding for the C5a peptidase and Lmb, respectively, resulted in a decreased invasion of *S. agalactiae* into HBMEC. Decreased invasion was also seen in lmb mutant strains. Finally, host cell invasion was significantly blocked in competition experiments with either purified recombinant Lmb or a polyclonal antibody directed against the Lmb of *S. agalactiae*. The *S. agalactiae* scpB-lmb mutant induced an equal amount of the neutrophil chemoattractant IL-8 release in comparison to the wild-type.
Conclusion: Taken together, our studies support the conclusion that Lmb promotes invasion of *S. agalactiae* into HBMEC but does not play a role in IL-8 release from HBMEC.

O101 A proposed mechanism of action of glucocorticosteroids in severe sepsis

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Objective: To investigate the mechanism of action of glucocorticosteroids (GL) in severe sepsis (SS).
Methods: 0.1 ml of blood sampled from 33 patients within 24 hours of presentation of SS, was incubated with 0.1 ml of RPMI 1640 for 18 h at 37ºC in 5% CO_2 with 10 ng/mL of endotoxin (LPS) in the absence/presence of 10M of dexamethasone (DEX). Soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) and tumour necrosis factor (TNF)-α were estimated in supernatants by ELISA. Sepsis was induced in 60 mice, both wild-type (WT) and TNF-deficient (KO), by the intraperitoneal challenge of $7\log_{10}$ CFU of multidrug-resistant *Pseudomonas aeruginosa*. They were equally divided in the following groups: A, WT-controls; B, WT pre-treatment with DEX; C, KO-controls; D: KO pre-treatment with DEX. Survival was recorded for six animals of each group; another nine were sacrificed six hours post challenge for estimation of sTREM-1 in serum and of TREM-1 on cell membranes of neutrophils. Blood was also drawn form two KO mice and treated as blood of patients with SS.
Results: According to the effect of DEX on LPS-triggered blood, patients were divided in two groups: those where sTREM-1 was increased (n = 11) and those where sTREM-1 was decreased (n = 22). Death occurred in nil (0%) and 11 (50%) of them respectively ($p = 0.005$). The former patients survived longer than the latter (log-rank: 7.42, $p = 0.0065$). Median changes of sTREM-1 for them were +20.52 and −62.03 pg/mL ($p < 0.0001$) and of TNF-α −84.40 and −405.40 pg/mL respectively ($p = 0.048$). Results from the animal study are summarised in the Table. sTREM-1 after stimulation of blood from KO mice with LPS was 117.74 pg/mL; it was increased to 179.05 pg/mL after the addition of DEX.

	A	B	C	D
Median survival (hours)	18	18	18	>168
Median TREM-1 (%)	36.61	19.01	24.81	13.61
Median sTREM-1 (pg/mL)	751.14	261.51	565.83	423.28

Conclusions: Survival benefit for SS is observed when DEX promotes increase of sTREM-1 in LPS-triggered blood. The action of DEX is antagonised by TNF-α because (a) survival is extended for KO mice pre-treated with DEX; these mice have low TREM-1 and high sTREM-1, and (b) DEX stimulates production of sTREM-1 by KO blood. Findings are compatible with the existence of a protease mediating cleavage of TREM-1 from cell membranes; DEX behaves as agonist and TNF-α as antagonist. It might be hypothesised that the beneficial effect of GL in SS is seen for patients with low expression of the TNF gene.

Improving diagnosis in the microbiology laboratory

O102 Usefulness of analysis of the Vbeta repertoire of T cells for the early diagnosis of staphylococcal and streptococcal toxic shock syndrome

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Objectives: Staphylococcal and streptococcal toxic-shock syndrome (TSS) are severe illnesses associated with the production of superantigenic toxins, which activate specific fractions of the T-cell population by linking the Vbeta domain of the T-cell receptor. Each superantigenic toxin is associated with a characteristic Vbeta "signature" in vitro. We postulate that an early analysis of the Vbeta repertoire of T cells in vivo during TSS may facilitate the diagnosis and the introduction of antitoxinic agents.
Methods: We have prospectively included in 2005 and 2006 patients with streptococcal or staphylococcal TSS according to case definition. The Vbeta repertoire of T cells (24 Vbeta segments) was analysed by flow cytometry with fresh PBMC obtained in the first 48 hours of admission. The presence of each superantigenic toxin gene in *S. aureus* and *S. pyogenes* isolates were detected by PCR.
Results: Four patients with staphylococcal (three menstrual and one non-menstrual) and two patients with streptococcal TSS (one postpartal and another associated with *S. pyogenes* bloodstream infection) were included in the study.
In cases of menstrual *S. aureus* TSS, a Vbeta 2 expansion corresponding to the signature of TSST-1, was detected at admission. Corresponding isolates were positive for the gene encoding TSST-1. A Vbeta amplification corresponding to the signature of SEB was detected in the patient with staphylococcal non-menstrual TSS. The corresponding isolate was positive for the gene encoding SEB but also genes encoding SEA and SED. All patients received clindamycin, when cultures detected *S. aureus* (none received linezolid as all isolates were susceptible to methicillin), and the latter received intravenous immunoglobulins (IVIg).
In cases of *S. pyogenes* TSS, a Vbeta signature of SPEB was detected in the patient with the postpartal TSS. No Vbeta signature was detected in the other patient who developped severe disseminated intravascular

coagulation requiring limb amputations. The two corresponding isolates were positive for the gene encoding SPEB. All patients with streptococcal TSS received clindamycin and IVIg when cultures detected *S. pyogenes*.
Conclusion: The analysis of the Vbeta repertoire in patients with staphylococcal and streptococcal TSS may be a useful tool for the early diagnosis, apart in the case of associated severe bloodstream infection. Such analysis may facilitate the early introduction of anti-toxinic agents such as clindamycin, linezolid or IVIg.

O103 Microcalorimetry – a novel method for rapid diagnosis of bloodstream infections

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Background: Early detection of microorganisms in blood is essential for early diagnosis and treatment of bloodstream infections. Calorimetric detection of microbial growth may be more sensitive and rapid than blood culture systems. We compared microbial detection in spiked blood specimens using a commercial system (CO_2 detection by colour change) and an isothermal microcalorimeter.
Methods: *Escherichia coli* (ATCC 25922) or *Staphylococcus aureus* (ATCC 29213) were added at 3 different concentrations to anticoagulated blood obtained from healthy volunteers. Aliquots of 7.5 ml spiked blood were added into aerobic blood culture bottles containing 40 ml growth medium and incubated in the blood culture instrument (BacT/ALERT, bioMérieux, Durham, NC, USA). Before incubation, 1 ml of the bottle content was inoculated in glass ampoules containing 2 ml sterile trypticase soy broth for calorimetry (TAM III, Thermometric AB, Järfälla, Sweden). Blood cultures and calorimetry ampoules were simultaneously loaded in corresponding instruments and incubated at 37ºC for 72 hours. Time to positivity was defined as the interval from incubation start until positive signal (BacT/ALERT) or until the heat flow rate increased $\geqslant$10 µW above baseline (calorimeter detection limit = ~0.3 µW).
Results: Time to positivity was considerably shorter using calorimetry (Table). Blood without added pathogens produced no heat signal above baseline. Spiked specimen heat signals rose to >100 µW as the pathogens multiplied during the incubation period.

Pathogen (cfu/mL blood)	Time to positivity for BacT/ALERT (h)	Time to positivity for calorimeter (h)	Peak heat flow (µW)
E. coli (10^5)	8	3.2	761
E. coli (10^3)	11.8	5.4	733
E. coli (10^1)	12.2	7.3	741
S. aureus (10^5)	10.5	4.4	278
S. aureus (10^3)	17	7.8	244
S. aureus (10^1)	20.8	12.9	114

Conclusion: Microcalorimetry has the potential to detect bloodstream infections earlier with a smaller volume of blood than commercial blood culture systems and for routine use in microbial screening of blood cultures.

O104 A novel chromogenic medium for vancomycin-resistant *Enterococcus faecium* and *Enterococcus faecalis* detection

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Objectives: Vancomycin-resistant enterococci (VRE) are widespread worldwide. Since August 2004, several wards at the Bicetre hospital faced a clonal outbreak of a vancomycin-resistant *Enterococcus faecium* strain BCT-1 expressing an heterogeneous VanD-phenotype vanA genotype (Naas et al., 2005, JCM, 43, 3642). Rapid identification of VRE carriers from surveillance cultures is crucial in order to control the VRE spread. We assessed a new selective chromogenic medium VRE ID (bioMérieux, France) that enables isolation and presumptive identification of VRE directly from rectal swab samples.
Methods: Within a month 191 patients totalising 199 rectal swabs were evaluated for VRE colonisation. Rectal swabs were first grown for 18 h in a bile broth (AES, France) containing 3 µg/mL vancomycin, at 37ºC, prior to plating on either the conventional Enterococcosel-Agar-Vancomycin medium (BD, France) or the chromogenic VRE ID medium. Suspect colonies growing on each media were identified and tested for glycopeptide susceptibility. Molecular characterisation of resistance genes and comparison of the strains (pulse field gel electrophoresis [PFGE]) were performed.
Results: A total of 11 VRE isolates (all *E. faecium*) were recovered from 199 specimens (17.7%). After 48h of incubation these VRE isolates were all recovered on each agar medium (sensitivity 100% for both); the positive predictive values were 20.8% and 91.7% for Enterococcosel Agar Vancomycin and VRE ID, respectively. PFGE revealed that these *E. faecium* isolates were undistinguishable or clonally related to the epidemic clone. All of them were of vanA genotype.
Conclusions: Chromogenic VRE ID medium showed higher specificity to the conventional method for detection of VRE. The chromogenic and selective characters of VRE ID medium enhanced recovery and identification of VRE and reduced unnecessary confirmations and time-consuming tests.

O105 Evaluation of a rapid test panel, the API Strep 20, the BD Phoenix and VITEK 2 automated instruments, and Raman spectroscopy for species identification of Enterococci

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Objectives: The identification of vancomycin-resistant enterococci (VRE) has become an important component of infection-control programmes. The differentiation between vanA/B-VRE [high-level and transferable vancomycin resistance in *Enterococcus faecium* and *Enterococcus faecalis* (Efe/Efa)] and vanC-VRE [intrinsic low-level vancomycin-resistance in *Enterococcus casseliflavus* and *Enterococcus gallinarum* (Ecas/Egal] is relevant since in contrast to vanA/B-VRE, vanC-VRE have not been implicated in outbreaks. Furthermore, vancomycin treatment failure has been associated with infections caused by vanC-VRE. Differentiation of vanC-VRE from other enterococcal species by adequate identification is therefore relevant since low-level resistance may not be detected using the CSLI breakpoints.
In the current study we evaluated a simple rapid test panel (RTP), the BD Phoenix and VITEK 2, the API Strep 20 and Raman spectroscopy for their accuracy to identify clinical relevant enterococcal species and their sensitivities and specificities to distinguish Efe/Efa from vanC positive enterococci.
Method: In total 96 clinical enterococcal strains comprising 8 different species were analysed. A genotypic test based on the sequence of the rpoA gene was used as reference method. The phenotypic test panel provided a species identification within 4 hours, testing the reduction of litmus milk, acidification of arabinose and methyl-alpha-D-glucopyranoside, hydrolysis of L-arginine, pigment production and motility. Raman spectroscopy is a relatively new identification method under development yielding results within 1 minute.
Results: The table shows the accuracy of the different tests relative to the sequence-based identification varied between 86% (BD Phoenix) and 96% (Raman). The best method to distinguish Efe/Efa from vanC positive species was the RTP with a sensitivity and specificity of 100% The API method performed poorly with a relative high sensitivity of 98.6% but very low specificity (41.7%). With this method 7/12 Ecas/Egal were identified as *E. faecium*.
Conclusions: All methods were comparable regarding the identification of enterococci. However, from the routine laboratory tests the RTP was the most rapid and reliable method to distinguish Efe/Efa from Ecas/Egal. Raman spectroscopy is a very promising fast alternative.

The API revealed a high percentage of false positive *E. faecium* identifications, which may result in unnecessary infection control interventions.

Identification method	Total no. (%) accurate	Discrimination between Efe/Efa and Ecas/Egal	
		Sens. (%)	Spec. (%)
Rapid phenotypic tests	88 (92)	100	100
API Strep 20	35 (39)	93.6	41.7
BD Phoenix	83 (86)	98.4	100
VITEK 2	84 (88)	98.5	91.7
Raman spectroscopy[a]	244 (96)	99.5	36.1

[a]A selection of 85 strains were tested in triplo.

O106 Rapid identification of viridans streptococci by mass-spectrometric discrimination

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Objectives: Viridans streptococci (VS) are responsible for several systemic diseases such as endocarditis, abscesses and bacteraemia. Conventional identification of these organisms is laborious and not always reliable. The aim of the present study was to evaluate the use of intact cell matrix-assisted laser desorption time-of-flight mass spectrometry (MALDI-TOF-MS) for rapid identification of 10 different species of VS.
Methods: A total of 99 VS strains were analysed with MALDI-TOF-MS. As MALDI-TOF reference database for each of the 10 different species (*S. intermedius*, *S. mutans*, *S. oralis*, *S. parasanguinis*, *S. salivarius*, *S. sanguinis*, *S. mitis*, *S. constellatus* ssp. *constellatus*, *S. anginosus* and *S. gordonii*) one reference strain (DSMZ, Braunschweig, Germany) and 3 reliable isolates from our reference stock were used. To evaluate the mass-spectrometric discrimination results all strains were identified parallel with phenotypic (rapid ID 32 STREP system) and genotypic (species specific PCRs and sequence analysis of the 16 S rRNA gene) methods.
Results: The MALDI-TOF-MS identified 71 strains as *mitis* group (37 *S. oralis*, 23 *S. mitis*, 10 *S. parasanguinis*, 1 *S. sanguinis*, 0 *S. gordonii*); 23 as *anginosus* group (19 *S. anginosus*, 2 *S. intermedius*, 2 *S. constellatus*) and 5 *S. salivarius* strains. The comparison of the species identification results of the MALDI-TOF analyses and the phenotypic/genotypic identification systems showed a consistency of 100% on species level.
Conclusions: The results of the present study showed that mass-spectrometry might be used as a rapid method for identification of VS from clinical samples. The method was able to discriminate reliably species of the rather homogeneous mitis group, which is still difficult with genotypic or phenotypic identification methods.

O107 Circulating *Candida* antibodies precede invasive candidiasis in patients with haematological malignancy

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Objectives: Antibodies (Ab) are generally not considered useful for the early diagnosis of invasive fungal infection. We studied the kinetics of *Candida* antigen (Ag) and Ab in patients with haematological malignancy using two systems commercially available in Europe (Platelia Candida EIA and Platelia Candida Ab/Ac/Ak, Marnes-La-Coquette, France, and Serion ELISA Antigen Candida and Serion ELISA Classic *Candida albicans* IgG/IgM/IgA, Institut Virion\Serion GmbH, Würzburg, Germany).
Methods: Serum or plasma samples were available from 21 haematology patients (242 samples) with proven invasive candidiasis (IC) collected between first admission and 253 days after the candidaemia. The control group consisted of 35 patients who had undergone HSCT but without evidence for IC (135 samples). Ab and Ag were determined using 2 detection systems (Biorad and Serion), and the reactivity was related to *Candida* colonisation, mucositis and number of days of neutropenia.
Results: Ab and Ag were detected in more samples from patients with IC as compared to the control group for both systems ($P < 0.05$). Ab was detected at a median of −23 (Biorad) and −20 (Serion) days before positive blood culture as opposed to −1 and −11 days, resp., for Ag. Ab was detected more frequently in patients with >10 days of neutropenia (corresponding with multiple episodes of neutropenia) as compared to those with <10 days of neutropenia ($P < 0.05$). When analysing individual patients Ab were first detected following a previous episode of neutropenia, suggesting subclinical IC. In the control group the Biorad test was negative in 91.1% (Ag) and 93.3% (Ab) of samples and the Serion test in 93.3% (Ag) and 97.6% (Ab). Reactivity in this group did not correspond with colonisation or mucositis.
Conclusion: *Candida* Ab are very early markers of IC in haematology patients who have undergone multiple courses of chemotherapy. These data suggest that subclinical invasive *Candida* infection causes an Ab response and precedes clinically manifest invasive *Candida* disease.

O108 Evaluation of the increase rate of MRSA detection using enrichment

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The objective of the work carried out was to determine the increase rate of MRSA detection using an enrichment step compared to direct culture on chromogenic agar.
MRSA screening swabs from neonates in the NICU were compared: each swab was first cultured directly onto chromogenic MRSA ID agar and then incubated in Brain Heart Infusion broth for 24 hours. The broths were subsequently subcultuerd onto MRSA ID agar. All plates were incubated for 48 hours aerobically at 37ºC and checked for the presence of green colonies, which is indicative for MRSA, at 24 and 48 hours. All green colonies had confirmatory tests for MRSA. The limit of detection was determined for both direct and enriched cultures on MRSA ID agar using doubling dilutions up to 1/4096 from an initial 0.5 McFarland suspension. Plates were incubated for 24 hours aerobically at 37ºC after which colony counts were performed.
A total of 1181 swabs were tested using direct and enriched culture methods. With direct culture, 16 isolates of MRSA were detected after 24 hours incubation and a further 6 after 48 hours incubation (22 in total). Following enrichment, 34 isolates were detected after 24 hours incubation and one more following 48 hours incubation (35 in total). Enrichment yielded a 59% increase in the number of isolates of MRSA detected compared to direct culture. The limit of detection for direct culture was 69,000 cfu/mL at 1/128 dilution with 84,000 cfu/mL at 1/2048 dilution following enrichment.
Results showed that the limit of detection was higher following enrichment indicating a 16-fold increase in sensitivity compared to direct culture. The comparison of patient samples correlated with this by showing an increase of 59% in the rate of detection of MRSA following enrichment. In conclusion enrichment has proven to dramatically increase the sensitivity and rate of detection of MRSA compared to direct culture alone and in order to ensure maximum sensitivity in the detection of MRSA enrichment should be employed as the method of choice in routine laboratories.

O109 Phenotypical expression of extended spectrum β-lactamases in Enterobacteriaceae: prevalence in 3 university hospitals in Germany, 2003–2006 and implications for screening

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Objectives: Resistance of Enterobacteriaceae (EB) to third-generation cephalosporines has increased during the recent years. Especially strains

expressing extended spectrum β-lactamases (ESBL) limit therapeutic options. The CLSI has established recommendations for phenotypical screening of ESBL in *Klebsiella* and *E. coli*. However, similar standards for ESBL in other EB are missing. Using the data set of the German Network for Antimicrobial Resistance Surveillance (GENARS) we assessed the prevalence of ESBL in selected species of EB in order to estimate applicability for screening in EB other than *Klebsiella* and *E. coli*.

Methods: Analysis is based on first isolates of EB collected from Jan 2003 to Sept 2006 in 3 university hospitals. Antimicrobial susceptibility is determined as Minimal Inhibitory Concentration by the automated test system Micronaut® with microdilution panels for several ESBL test substrates including ceftazidime (CAZ), cefotaxime (CTX), cefpodoxime proxetil (CPP) and cefpodoxime/clavulanic acid (CPC). According to CLSI guidelines, these antibiotics are used as indicators for (a) ESBL screening: CAZ ⩾2 mg/L or CTX ⩾2 mg/L or CPP ⩾8 mg/L and (b) ESBL phenotypical confirmation: ratio of CPP and CPC ⩾8 in *E. coli* and *Klebsiella* spp. With this procedure the presence of a K1 β-lactamase of K. oxytoca is phenotypically excluded.

Test results for ESBL screening and confirmation and indices for ESBL screening by species in Enterobacteriaceae (n = 28,990) in Germany 2003–2006[a]

Species	ESBL Phenotype				N	ESBL screening indices (%)			
	scr+ cnf+	scr− cnf+	scr+ cnf−	scr− cnf−		prev	sens	spec	ppv
K. oxytoca	83	39	36	1,515	1.673	7.3	68.0	97.7	69.7
K. pneumoniae	172	12	119	2,696	2,999	6.1	93.5	95.8	59.1
E. coli	298	28	334	13,059	13,719	2.4	91.4	97.5	47.2
P. mirabilis	31	4	75	2,397	2,507	1.4	88.6	97.0	29.2
Citrobacter spp.	11	5	116	700	832	1.9	68.8	85.8	8.7
Serratia spp.	5	2	89	527	623	1.1	71.4	85.6	5.3
Enterobacter spp.	22	4	478	1,133	1,637	1.6	84.6	70.3	4.4
C. freundii	10	0	237	582	829	1.2	100.0	71.1	4.0
E. cloacae	20	0	774	1,377	2,171	0.9	100.0	64.0	2.5
S. marcescens	2	0	118	636	756	0.3	100.0	84.4	1.7
M. morganii	5	1	421	217	644	0.9	83.3	34.0	1.2
Total	659	95	2,797	24,839	28,390				

[a]scr: screening; cnf: confirmation; prev, prevalence; sens, sensitivity; spec, specificity; ppv: positive predictive value.

Results: Data of 28,390 isolates were analysed (see the table). Proportions of confirmed ESBL are highest in *K. pneumoniae* (5.7%) followed by *K. oxytoca* (5.0%) and *E. coli* (2.2%) compared to 0.3% to 1.3% in the remaining EB. Isolates with positive confirmatory ESBL test and negative screening occur in all species, most prominently in *K. oxytoca* (2.3%). The proportion of non-confirmed positive screenings ranges from 2.2% to 4% in *E. coli*, *Klebsiella* spp. and *P. mirabilis* compared to 13.9% to 65.4% in other EB. In the study population the positive predictive value is highest for *K. oxytoca* (69%), *K. pneumoniae* (59%) and *E. coli* (48%) and below 30% for all other EB.

Conclusion: Phenotypical ESBL screening according to CLSI guidelines indicate a lack of sensitivity for K. oxytoca. Therefore, new screening algorithms for detection of resistance due to β-lactamases have to be created and should probably include genotypical properties in EB.

O110 Predictive value of C-reactive protein and procalcitonin for bacteraemia in adult community-acquired infection

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Objectives: The value of C-reactive protein (CRP) and procalcitonin (PCT) in predicting bacteraemia has not been compared in a clinical model based on variables from clinical assessment and routine laboratory tests.

Methods: Eighty-four bacteraemic and 474 non-bacteraemic adult patients with community-acquired infections were prospectively studied. A basic model of septic evaluation integrating routine clinical and laboratory variables, and infection site was developed and analysed for its predictive value alone and with further incorporation of either CRP or PCT. The area under the receiver-operating characteristic curves (AUC) of the CRP-incorporated and PCT-incorporated model were compared to the AUC of the clinical model.

Results: The independent predictors of bacteraemia identified in the clinical model were age ⩾ 60 years-old (OR 1.80; 95% CI: 1.03–3.14), presence of fever (OR 2.56; 95% CI: 1.09–5.97), body temperature higher than 38.3ºC at blood culture (OR 2.23; 95% CI: 1.20–4.13), hypotension (OR 3.83; 95% CI: 1.23–11.99), heart rate ⩾120 beats/min (OR 2.34; 95% CI: 1.29–4.22), leukocyte count ⩾ 15,000 cells/μL (OR 2.15; 95% CI: 1.12–4.12), lymphopenia (OR 5.89; 95% CI: 3.17–10.96), thrombocytopenia (OR 2.36; 95% CI: 1.33–4.21), hepatobiliary tract infection (OR 8.43; 95% CI: 3.13–22.7), urinary tract infection (OR 2.41; 95% CI: 1.24–4.69), and orthopaedic site infection (OR 56.64; 95% CI: 6.14–522.52). The value of the AUC in the clinical model was 0.829 (95% CI: 0.781–0.878). Adding CRP level to the basic clinical model did not change any of the identified predictors. In the PCT-incorporated model, PCT ⩾0.5 ng/mL became a new independent predictor (OR 4.69; 95% CI: 2.62–8.41) and increased the value of the AUC to 0.848 (95% CI: 0.803–0.894). However, the difference between the AUC of the clinical model and the PCT-incorporated model was not significant (P = 0.388).

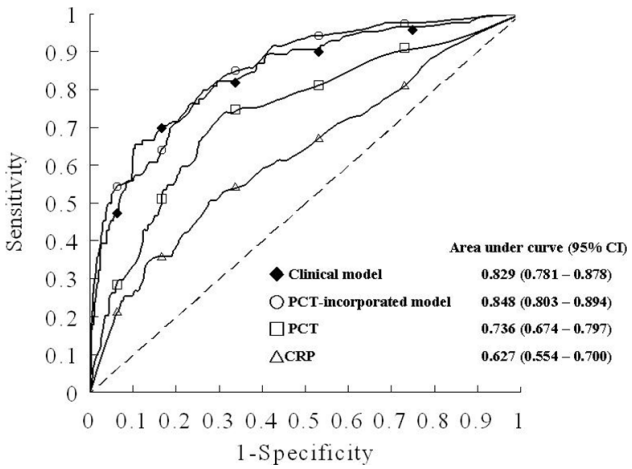

Figure 1. Area under the receiver operating characteristics curves of the clinical model, PCT-incorporated model, and univariate analyses with procalcitonin (PCT) and C-reactive protein (CRP) for the prediction of bacteraemia.

Conclusion: The limited additional help of the inflammatory markers CRP or PCT in bacteraemia prediction compared to a model of clinical assessment and routine laboratory information required for septic evaluation suggest the need for further justification of the use of these measurements in cost-constrained settings.

O111 A new chromogenic agar medium, chromID VRE®, to screen for vancomycin-resistant *Enterococcus faecium* and *Enterococcus faecalis*

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Objectives: Enterococcus species are members of the normal intestinal flora, and are the most common aerobic Gram-positive cocci found in the large bowel of humans. The organisms have gained more notoriety, however, as nosocomial pathogens, now grouped as the third most common blood borne pathogen in the United States. At least one factor accounting for this pathogenicity is their propensity to carry plasmid-mediated resistance markers to vancomycin. The development of reliable and rapid methods for the identification of patients colonised with vancomycin-resistant enterococci (VRE) is central to the prevention

of person-to-person transmission of this agent within the hospital environment. To this end, we conducted clinical trials for a chromogenic agar medium designed by bioMérieux (France) (chromID VRE) to recover VRE from stool and identify the isolated colonies as either *Enterococcus faecium* (VREfm) or *E. faecalis* (VREfs) based on different colony colour.
Methods: We compared the performance of this medium with bile esculin azide agar supplemented with vancomycin (BEAV). For this study, 120 stool samples were plated on each test medium and examined after 24 and 48 hours of incubation. Culture positive results were further identified using biochemical reactions (VITEK 2®), vancomycin susceptibilities, and PCR.
Results: At 24 hours, the sensitivity and specificity were: BEAV: 96%, 73%; chromID VRE: 90%, 89%. The positive predictive values for identification of positive samples by the chromogenic medium and BEAV at 24 hours were: chromID VRE 83%, BEAV 68%. Increased length of incubation (48 hr) did not significantly improve sensitivity, specificity, or positive predictive value for either medium. In addition, chromID VRE identified 2 isolates of VREfs that were not recovered using BEAV. Furthermore, the chromID VRE was capable of identifying patients colonised with both VREfm and VREfs – a feature useful for epidemiology follow-up that is not available with BEAV.
Conclusions: We conclude that this new and innovative chromogenic agar, chromID VRE, provides improved recovery of VRE from stool specimens and the added advantage of differentiation between VREfs and VREfm with an increase of specificity towards enterococci intrinsically resistant to vancomycin. Extended incubation beyond 24 hours did not significantly improve recovery of VRE and resulted in a decreased specificity.

Epidemiology of (CA-)MRSA

O112 Recent trends in epidemiology of MRSA in Finland

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Objectives: In Finland, a worrisome increase in the annual numbers of MRSA cases was observed from 2001 to 2004. Active control measures were implemented from 2005 to decrease MRSA cases. We report here the recent changes in MRSA epidemiology.
Methods: All MRSA isolates are notified by local laboratories to National Infectious Disease Register (NIDR) at National Public Health Institute (KTL) and tested at National Reference Laboratory at KTL by genotyping and antimicrobial susceptibility testing. Pulsed field gel electrophoresis (PFGE) was used as the primary genotyping method to distinguish different MRSA strains. In addition, multi locus sequence typing (MLST), staphylococcal cassette chromosome mec (SCCmec) analysis, and spa-typing were used to analyse representatives of each new epidemic MRSA strain. The clonal complex analysis was performed by BURP and eBURST programmes. The presence of PVL genes (lukS-PV – lukF-PV) was detected by PCR.
Results: The annual number of MRSA isolates reported to the NIDR rose over ten-fold, from 120 in 1997 to 1458 in 2004. In 2005 and 2006 (−30.9.2006) 1375 and 1004 new MRSA cases were detected, respectively. In general, the predominant MRSA strain types remained the same during 2004–2006: FIN-16, FIN-7, FIN-10, FIN-21 and FIN-4. However, in 2006 the number of FIN-7 strains showed an increasing trend whereas FIN-21 decreased from year 2005. Community-MRSA strains (FIN-4, FIN-7 and FIN-10) composed 30% of all MRSA cases in 2005. Eleven new epidemic MRSA strains have been identified since 2005. The number of cases within these outbreaks has been small. Four strains were resistant to B-lactam antibiotics only; the remainders showed resistance also to one or two other antibiotic groups. All except three strains were members of known MLST clonal complexes: CC5 (ST335, ST874, ST5, ST788), CC8 (ST8), CC45 (ST45) and CC59 (ST375). For one strain (ST849) no predicted founder could be identified and two strains (ST93 and ST853) were singletons. Four of the strains possessed SCCmec type IV, three SCCmec type V and one SCCmec type I. Three strains harboured a nontypeable SCCmec type.
Conclusions: The increasing trend in MRSA case numbers turned to a decline in 2005. Since then, several new epidemic MRSA strains have been detected. They differ from earlier strains as being non-multiresistant with SCCmec types IV, V or nontypeable. The outbreaks have been small and local, which speaks for the success of active control measures.

O113 Differences in the molecular epidemiology of methicillin-resistant *Staphylococcus aureus* within the Dutch–German EUREGIO MRSA-net Twente/Münsterland

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Objectives: The increasing cross-border transfer of patients and healthcare workers (HCW) within the EU causes enormous problems with regard to control the spread of multi-resistant pathogens. Especially within the Dutch–German border region, there are great differences in the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA): the Netherlands <1%, Germany ~5–25%. In addition, within the last two years an increasing number of community-acquired (CA)-MRSA could also be noted in the Netherlands, which represent an additional danger. The objective of this study was to elucidate the molecular cross-border epidemiology of MRSA within the EUREGIO Twente/Münsterland.
Methods: The first MRSA isolates of all patients and HCWs obtained within the EUREGIO MRSA-net Twente/Münsterland were typed. The epidemiological backbone of the project is a typing network providing a common language based on *S. aureus* protein A (spa) gene sequencing.
Results: Since the beginning of the project in July 2005, more than 1400 isolates from in- and out-patients and nursing home inhabitants were typed. Three spa types (t025, t091, t162) were detected only within the Euregio. The spa types t004, t009, t012, t024, t036, t051 and t084 were determined in the German part of the Euregio, only. However, the majority of spa types on the German side were in concordance to the national epidemiological trend with spa t001, t002, t003, t004 and t032 as the most common spa types. The spa type t044 – often associated with CA-MRSA in central Europe – was detected on both sides; 1% on the German and ~15% on the Dutch side of the border, underlining the emerging problem of CA-MRSA. Focusing on hospitalised patients, MRSA in blood cultures – only detected on the German side – were mainly spa type t001, t003, t004, t008 and t032.
Conclusion: In summary, the use of spa typing elucidated differences in the regional epidemiology of MRSA. However, cross-border spread of MRSA was also detected. Therefore, new co-ordinated regional and cross-border strategies have to be developed in the MRSA-net to fight against MRSA.

O114 Prevalence of methicillin-resistant *Staphylococcus aureus* in the southern and eastern Mediterranean – final results from the ARMed project

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Objective: The high prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) within the northern Mediterranean countries of Europe has already been documented. However, the concomitant situation in centres within the southern and eastern countries of the region was previously unknown. The Antibiotic Resistance Surveillance and Control in the Mediterranean Region (ARMed) project [www.slh.gov.mt/armed] provided a first time opportunity for a longitudinal multi-year study of trends of antimicrobial resistance amongst this species within these countries.
Methods: The ARMed project used comparable protocols to those adopted and validated by the European Antimicrobial Resistance Surveillance System (EARSS) and collected susceptibility test results

from invasive isolates of *S. aureus* routinely isolated from blood in 59 participating laboratories within Algeria, Cyprus, Egypt, Jordan, Lebanon, Malta, Morocco, Tunisia and Turkey, who also took part in a external quality assurance programme throughout the project. The laboratories followed their routine procedures and breakpoints, which in 86.8% of the participants were based on CLSI (formerly NCCLS) guidelines. MRSA incidences at country level for 2005 were calculated by combining resistance data from the ARMed database with information on total number of patient days on the basis of hospital codes.
Results: A total of 5,353 *S. aureus* isolates were reported to ARMed for the whole study period (2003–2005), with an average MRSA proportion of 38%. MRSA incidence ranged from 4.61 per 100,000 patient days in Morocco to 18.37 in Egypt. Laboratories in Egypt, Jordan, Cyprus and Malta reported prevalence in excess of 50% in at least two out of the three study years. Furthermore, over the study period, significant increases were observed in Egypt (33% to 63%) and Malta (43% to 55%) whilst decreases were reported in Jordan (66% to 32%) and Turkey (43% to 35%).
Conclusion: ARMed resistance data has improved the MRSA epidemiological picture within the whole Mediterranean area and confirmed that the region as a whole exhibits a high prevalence. Other data from the whole region collected by ARMed as well as by other projects appears to suggest that antimicrobial consumption together with sub-optimal infection control infrastructure and activities may be important drivers behind this high prevalence situation and merit further research in the future.

O115 Molecular epidemiology of non-multiresistant and community-associated MRSA in Geneva, Switzerland, over a 13-year period

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To evaluate the molecular epidemiology of methicillin-resistant *Staphylococcus aureus* strains differing from the multiresistant, healthcare-associated clone endemic in Geneva, we identified two collections of 151 non-multiresistant strains over a 13-year period. Extensive characterisation included staphylococcal chromosome cassette mec and accessory gene regulator type determinations, toxin content, as well as genotyping. Our collection contained an extraordinary diversity of strains in terms of genome content, including internationally well-known isolates. Fifty-eight percent (n = 92) of strains contained one important toxin. A large proportion harboured a SCCmec IV (n = 75) or V cassette (n = 7). The most important clusters were composed of PVL positive ST80 (n = 39), ST5 containing PVL or TSST (n = 15) and ST88 harbouring the exfoliatin A (n = 10). A homogeneous cluster of PVL positive, gentamicin-resistant strains (ST152 MRSA V) was found in 5 patients from Kosovo. Other strains showed previously uncharacterised genome contents (e.g. PVL negative ST1-MRSA-V) or profiles rarely observed in Europe (ST59 MRSA-IV or ST8-MRSA-IV). Several unusual clusters were detected, including one cluster in neonatology (PVL positive strain ST5 MRSA-IV) and transmission between 2 prison inmates (ST8 MRSA-IV). Our observation suggests that the molecular epidemiology of non multiresistant MRSA in the Geneva is very diverse and rapidly evolving through constant importation and community transmission of new strains.

O116 Community-onset MRSA bacteraemia: characteristics of patients presenting to hospital

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Objectives: To examine the distribution of patients with MRSA bacteraemia detected within 2 days of presentation in acute NHS hospitals in England from an analysis of approximately 7000 MRSA bacteraemias reported between October 2005 and September 2006.
Methods: In October 2005, an enhanced mandatory MRSA bacteraemia surveillance system (MESS) was introduced, including collection of additional data such as patient socio-demographic characteristics (e.g. age, gender, patient location), Trust characteristics (e.g. region, Trust type/size, location) and patient clinical characteristics (e.g. timing of MRSA bacteraemia detection, source of admission, specialty under which MRSA was detected). A subset of data on patients' risk factors is also collected and includes variables such as source of bacteraemia and previous contact with the health delivery system. Data were extracted for the period October 2005 to September 2006 from the MESS database, representing approximately 7000 reported MRSA bacteraemias in 172 acute NHS Trusts. Standardised screen formats were used to record the dates of admission and onset of bacteraemia, location of patient prior to admission, location and specialty at the time of diagnosis.
Results: A significant proportion of MRSA bacteraemias (34%) were detected within 2 days of presentation to the Trust. More specifically, 9% were detected at presentation to the Trust and a further 25% within 2 days of admission. Of those detected on presentation (and not admitted), over one third (44%) were seen at Accident and Emergency and a further 14% were regular attendees, e.g. at renal units. Most patients with MRSA bacteraemia detected within 2 days of presentation to the Trust were admitted from home (66%), 16% came from nursing homes and 7% from other Acute Trust hospitals. Data on patients' risk factors indicate that skin and soft tissue infections and UTIs are the most common risk factors for developing MRSA bacteraemia within 2 days of presentation.
Conclusion: Preliminary results appear to support the hypothesis that a large proportion of patients presenting with MRSA bacteraemia at admission have had prior healthcare interactions in the preceding three months. Identification of readmission patterns may provide a useful means of targeting interventions to prevent some of these MRSA bacteraemias, whether originating from previous hospital admissions or from nursing homes in the community healthcare setting.

O117 Heteregeneous but higher adhesive properties of hospital-acquired MRSA Lyon clone isolates in comparison to MSSA isolates

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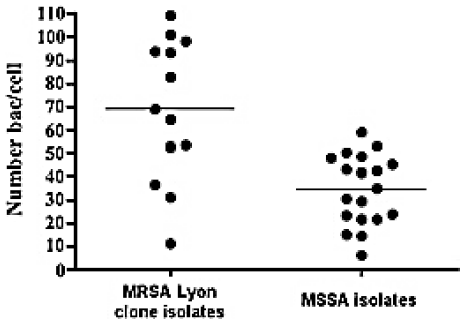
Objectives: The success of pandemic hospital-acquired MRSA clones may be due to high adhesive properties. Adhesion is mediated by various different cell wall proteins called adhesins that are tightly regulated. The accessory gene regulator (agr) is one acting regulator. agr dysfunction is associated with overexpression of certain adhesins and seems to be responsible of the success of the CMRSA-3 clone in Canada. In this way, we compared adhesive properties, the adhesin gene content and the functionality of agr of MRSA Lyon clone isolates to those of MSSA isolates.
Methods: We studied *S. aureus* isolates responsible for bloodstream infections in intensive care units in 2003. Nineteen isolates were susceptible to methicillin and 13 MRSA isolates had characteristics of the MRSA Lyon clone (sequence type 8, agr allele type 1 and positive for the sea gene). The binding behaviour onto airway epithelial cells (HAECs) were determined by quantifying the number of adherent bacteria per cell (30 cells per strain were used). The adhesin gene profile (bbp, can, eno, ebpS, fib, clfA, clfB, fnbA and fnbB) was determined with multiplex PCR for each isolates. The dysfunction of agr was detected by the absence of delta-haemolysin production.
Results: The number of *S. aureus* isolates per cell was more homogeneous for MSSA isolates than MRSA isolates, but the adhesion of MRSA isolates was significantly higher than MSSA isolates (68.94 $\pm$ 8.483 and 34.31 $\pm$ 3.446, respectively, $p < 0.001$) (Figure). MRSA isolates were all positive for eno, fib, clfA, clfB, fnbA and fnbB, only. MSSA isolates were all positive for eno, clfA, clfB and fnbB and inconstantly positive for others. The absence of delta-haemolysin production tended to be more frequent among MRSA isolates (7/13 versus 16/19 isolates), but the difference was not significant ($p = 0.11$). No correlation between the adhesive property and the absence of delta-haemolysin production was observed, both in MRSA and in MSSA isolates.

Conclusion: We demonstrated that adhesive properties onto HAECs of MRSA Lyon clone isolates was higher than that MSSA isolates, but was heterogeneous despite similar genetic background and adhesin gene profile. This could be the result of overexpression of adhesins independently of an agr dysfunction.



O118 Community-associated MRSA ST8-SCCmecIVa (USA-300): experience in England and Wales

A.M. Kearns, M. Ganner, R.L.R. Hill, C. East, I. McCormick Smith, M.A. Ganner, M.J. Ellington (London, UK)

Objectives: Although many distinct genetic lineages of community-associated MRSA (CA-MRSA) have been reported worldwide, one clone has proved highly successful in the USA. The ST8-SCCmec IVa (or so-called USA-300) clone has been associated with community outbreaks, nosocomial transmission, and identified as the primary cause of skin and soft tissue infections (SSTI) presenting to Emergency Departments across the USA. Following enhanced surveillance for CA-MRSA, and in the light of these observations, we have mapped the epidemiology of this clone in England and Wales.
Methods: Over a 2 year period (2004/5), all MRSAs referred to the national Staphylococcus Reference Unit for England and Wales were characterised microbiologically. Isolates identified as CA-MRSA were subjected to detailed characterisation (including MLST, spa typing, toxin gene profiling, PFGE and antibiotic susceptibility testing). Epidemiologic, demographic and clinical data were also collated.
Results: Of ca. 300 CA-MRSA identified, 40 belonged to the ST8-SCCmecIVa clonal lineage; the third most common CA-MRSA occurring nationally. All 40 isolates were PVL-positive, spa t008 and belonged to agr group 1. Nine different sub-types were distinguishable by PFGE; almost half belonged to a single pulsotype. All were resistant to β-lactams; resistance to erythromycin, ciprofloxacin, gentamicin, tetracycline and fusidic acid was variable. All were susceptible to clindamycin, trimethoprim, vancomycin, linezolid and mupirocin. The majority of patients (32, 80%) were community-based, including 23 males and 17 females; ages ranged from <1 to 89 y (median 25 y). 60% presented with skin infections (e.g. boils, abscesses or insect bites) and 25% with soft tissue infections. One patient died. Most cases were sporadic, but 4 community-based clusters were identified involving MSMs, household contacts and individuals attending the same GP surgery. Eight patients gave a history of foreign travel (e.g. the USA), the remainder were acquired domestically.
Conclusion: The ST8-IVa lineage of CA-MRSA has been occurring in England and Wales since at least 2002. Whilst numbers remain small, the occurrence of community-based clusters and the death of one patient give cause for concern. The emergence of resistance to multiple classes of antimicrobials allied to evidence of clonal expansion highlight the need for continued surveillance to underpin effective therapeutic and infection control strategies.

O119 Community MRSA ST8 ("USA300") has arrived in Central Europe

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Objective: Community MRSA (cMRSA) "USA300", MLST ST8 was reported as particularly epidemic in Northern America. This cMRSA exhibits a few characteristics by which it can be detected easily. Here we report about characterisation of MRSA exhibiting spa sequence type t008 (congruent with MLST ST8) from community and nosocomial infections in Germany in order to assess the emergence and spread of the epidemic community MRSA "USA 300" in Central Europe.
Methods: Multilocus sequence typing (MLST), grouping of SCCmec elements by means of PCR, PCR demonstration of lukS-lukF arcA (arginindeiminase, indicator for ACME-island; msrA (macrolide efflux), mphB (macrolide phosphotransferase), and fosB.
Results: MRSA exhibiting t008, MLST ST8 which are exhibiting resistance to oxacillin and to ciprofloxacin but lacking lukS-lukF, arcA, msrA, mphB are known from community acquired wound infections and nasal colonisation in the Stuttgart area since 2000. Community acquired MRSA exhibiting t008, MLST8 and containing lukS-lukF, arcA, msrA, mphB have been recorded from deep seated infections of skin and soft tissue in Saarland, Rhineland-Pfalz, North-Rhine-Westphalia, and lower Saxony as well as in Tyrol (Austria) since 2004. In two cases spread to other patients occurred after admission of affected patients to surgical wards. ArcA, msrA, mphB are characteristic for community MRSA "USA 300" and were not detected in representatives of epidemic hospital MRSA and also not in other clonal lineages of lukS-lukF containing community MRSA.
Conclusion: lukS-lukF containing MRSA carrying other acquired genes which are characteristic for MRSA "USA 300" have been recorded from several locations in Germany. For reliable early warning MRSA exhibiting spa t008 should be further characterised for containing lukS-lukF, arcA, and mphB.

O120 Characteristics of complicated skin and skin structure infections due to staphylococci and the presence of Panton-Valentine leukocidin

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Objectives: An association has been proposed between Panton-Valentine leukocidin (PVL) and virulence in methicillin-resistant *Staphylococcus aureus* (MRSA) causing skin or respiratory tract infections. In a multicentre trial comparing ceftobiprole (BPR), an investigational cephalosporin, to vancomycin (VAN) in patients with complicated skin and skin structure infections (cSSSI), PVL-positive *S. aureus* characteristics were analysed and assessed for both MRSA and methicillin-susceptible (MSSA) isolates.
Methods: *S. aureus* isolates were analysed for PVL by multiplex PCR (n = 415). In vitro susceptibility (CLSI methodology) and a mecA probe were used to identify MRSA. Infection type was categorised as abscess, wound, or cellulitis. Infections with a depth either to the fascial plane or muscle were considered deep infections.
Results: Of 784 patients enrolled, *S. aureus* was identified in 494 baseline isolates from pus, leading edge of cellulitis, or debrided tissue. PVL was significantly more common in abscesses compared to wound infections or cellulitis among both MRSA ($P < 0.001$) and MSSA infections ($P < 0.005$). There was a trend for higher WBC counts among PVL-positive MRSA and MSSA isolates compared to PVL-negative MRSA and MSSA isolates. PVL-positive MRSA infections were associated with significantly lower rates of fever ($P < 0.001$) and lower levels of C-reactive protein (CRP) ($P < 0.005$) compared to PVL-negative MRSA infections. Lower overall cure rates were observed in PVL-positive MRSA infections (overall 89.3%; BPR 93%; VAN 85%). Overall cure rates for PVL-negative MRSA and PVL-positive or negative MSSA were >94% in both treatment arms.

	% MRSA		% MSSA	
	PVL(+)	PVL(−)	PVL(+)	PVL(−)
Infection type, % abscess	70.5 (67/95)*	26.3 (15/57)	68.0 (68/100)*	48.2 (79/164)
Deep infections, %	33.6 (32/95)	38.6 (22/57)	26.0 (26/100)	34.1 (56/164)
WBC > 10^4, %	32.2 (28/87)	21.3 (10/47)	41.4 (36/87)	31.0 (45/145)
Temperature >38ºC	14.7 (14/95)*	42.1 (24/57)	44.0 (44/100)	40.2 (66/164)
CRP > 50 mg/dL	28.0 (26/93)*	51.9 (28/54)	36.2 (34/94)	41.4 (67/162)

Conclusion: PVL-positive MRSA and MSSA were widely prevalent in cSSSI. The presence of PVL in both MRSA and MSSA was significantly associated with abscess compared to wound infections or cellulitis in this cSSSI trial. Lower fever and CRP levels in patients with PVL-positive MRSA infections may indicate impaired host responses to PVL-positive *S. aureus*. The presence of PVL was associated with lowest cure rates in patients with MRSA infections; however, cure rates in patients treated with BPR exceeded 90%.

Clinical trials of antimicrobials

O140 Micafungin versus caspofungin in patients with invasive candidiasis or candidaemia: a Phase III, randomised, double-blind, parallel group, non-inferiority study

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Objectives: To determine the efficacy and safety of two doses of intravenous micafungin (MICA) versus caspofungin (CAS) as an antifungal treatment for patients with confirmed invasive candidiasis or candidaemia.
Methods: Patients aged ⩾ 18 years with confirmed invasive candidiasis or candidaemia were randomised 1:1:1 to receive MICA 100 mg/day (MICA100), 150 mg/day (MICA150) or CAS (70 mg on day 1 and 50 mg/day thereafter). The primary efficacy endpoint was treatment success by investigators' assessment at the end of blinded intravenous therapy, defined as a positive clinical response and a positive mycological response. Patients who died during therapy or had missing values were considered treatment failures. Non-inferiority was defined as the lower bound of 95% CIs for treatment difference for MICA versus CAS exceeding −15%. Secondary efficacy endpoints included treatment success as assessed by the data review panel, clinical response, mycological response, incidence of emergent invasive fungal infections throughout the study and incidence of relapse post-treatment.

Treatment success	MICA		CAS
	100 mg	150 mg	
Overall, n (%)	147/199 (73.9)	142/202 (70.3)	137/192 (71.4)
Treatment difference vs. caspofungin, % (95% CI[a])	2.5 (−5.9, 11.0)	−1. 1 (−9. 3, 7.8)	

[a] Adjusted for baselin APACHE Il score and geographic region.

Results: The full analysis set (FAS) was the primary analysis set for efficacy and comprised 593 patients: 199 receiving MICA100, 202 receiving MICA150 and 192 receiving CAS. Overall treatment success was seen in 73.9% of MICA100, 70.3% of MICA150 and 71.4% of CAS patients. The two-sided 95% CIs exceeded the predefined non-inferiority margin of −15%; both MICA doses were therefore non-inferior to CAS. The data review panel assessments confirmed this result. On secondary efficacy endpoints, the three treatment arms were similar in terms of clinical and mycological response, and there were no significant differences in the incidence of emergent fungal infections during the study and in the incidence of relapse post-treatment. There were no clinically significant differences between the three treatment arms in terms of adverse events, including those of special interest, such as hepatic and renal function adverse events. The incidence of death during treatment was similar across treatment groups.
Conclusion: MICA100 and MICA150 were equally effective and were non-inferior to CAS on the primary efficacy endpoint of treatment success as defined by the investigator. There were no significant differences between the MICA treatment arms and CAS on all secondary endpoints. All three treatment regimens had similar safety profiles.

O141 Micafungin versus liposomal amphotericin B (AmBisome®) in paediatric patients with invasive candidiasis or candidaemia

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Objective: To compare the efficacy and safety of micafungin (MICA) with AmBisome® (L-AmB) in a paediatric subpopulation in a large, randomised, phase III trial in patients with invasive candidiasis or candidaemia.
Methods: In this double-blind, multicentre study, non-neutropaenic and neutropaenic paediatric patients aged ⩽15 years with clinical and microbiological evidence of invasive candidiasis or candidaemia were randomised 1:1 to receive intravenous MICA (2 mg/kg/day) or L-AmB (3 mg/kg/day) for a minimum of 14 days. All randomised patients were included in the full analysis set (FAS). Efficacy analyses were also performed for the per protocol set (PPS), which included all patients who had confirmed invasive candidiasis or candidaemia but did not have a further invasive infection caused by a non-*Candida* fungal pathogen, for whom the investigator's assessment of overall treatment success at the end of therapy (EOT) was available, and who received at least five doses of study drug and did not receive a prohibited antifungal medication. The safety set included all FAS patients. Overall treatment success was based on clinical and mycological response at the end of therapy, as assessed by the investigator. The post-treatment follow-up period was 12 weeks.
Results: A total of 106 patients (52 MICA and 54 L-AmB patients) were included in the FAS; 83 patients were included in the PPS. Age groups, including prematurity at birth, were well represented; candidaemia was the primary infection in the majority of patients in both treatment groups. The key efficacy results are shown in the table. The incidence of treatment-related adverse events (AEs) was lower with MICA than with L-AmB (36.5% versus 42.6%, respectively), as was the incidence of serious AEs (3.8% versus 9.3%, respectively). In addition, fewer patients receiving MICA than L-AmB discontinued therapy because of AEs (3.8% versus 16.7%, respectively).

	Treatment success, n (%)	
	MICA	L-AmB
FAS		
Overall	36/52 (69.2)	40/54 (74.1)
By neutropaenic status at baseline:		
<500 cells/μL	5/7 (71.4)	10/13 (76.9)
⩾500 cells/μL	31/45 (68.9)	30/41 (73.2)
PPS		
Overall	35/41 (85.4)	37/42 (88.1)
<500 cells/μL	5/5 (100)	9/10 (90.0)
⩾500 cells/μL	30/36 (83.3)	28/32 (87.5)

Conclusion: MICA was as effective as L-AmB in paediatric patients with candidaemia or invasive candidiasis, irrespective of neutropaenic status. Fewer MICA patients experienced treatment-related AEs, and there were fewer discontinuations due to AEs compared with L-AmB.

O142 Once daily, levofloxacin 750 mg for 5 days in the treatment of acute pyelonephritis and associated bacteraemia

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Objective: Urinary tract infections, in particular, pyelonephritis, have been identified as a common cause of Gram-negative bacteraemia. Studies that evaluated the prevalence of positive blood cultures in acute pyelonephritis (AP) yielded a blood pathogen in up to 23% of patients. The current clinical study represents one of the largest cohorts of patients with AP studied to date and provides an opportunity to assess the incidence, clinical presentation and outcome of bacteraemia in patients with AP.
Methods: This was a randomised, double-blind study. Subjects >18 yrs were stratified depending on diagnosis (cUTI or AP), residence, and catheter status. Subjects were randomised to treatment with iv/oral levofloxacin (levo) 750 mg qd, 5d or iv/oral ciprofloxacin (cipro) 400/500 mg bid, 10d. Blood cultures were obtained at study entry and, if positive, again at Posttherapy.
Results: 1109 subjects were enrolled; 1093 were randomised and received >1 dose of study drug; 619 had a confirmed clinical diagnosis and a study entry uropathogen (modified intent-to-treat [mITT] population). 192 mITT subjects were diagnosed with AP. Urine eradication rates for subjects with AP were 86.2% (81/94) for levo and 80.6% (79/98) for cipro (95% CI: −16.0, 4.9). 23 AP subjects (11 levo, 12 cipro) had a positive blood culture at study entry. 5 of the 23 subjects were hospitalised. All blood cultures identified *E. coli*. One cipro-resistant isolate (MIC > 32) was isolated from a cipro-treated subject. 9 levo subjects were clinical successes, 2 were failures. With cipro, 7 subjects were clinical successes, 2 were failures and for 3, the outcome was unknown. The urine pathogen was eradicated in 10 and was unknown for 1 levo subject compared to eradicated in 7, persisted in 3, and unknown in 2 cipro-treated subjects. A repeat blood culture was obtained for 7 levo and 8 cipro subjects, with the blood pathogen eradicated in all subjects. For one other cipro-treated subject, the blood pathogen was presumed persisted based on clinical failure. The outcome for the cipro-resitant *E. coli* was unknown.
Conclusions: For the overall population (cUTI and AP) and for the cUTI and AP cohorts. 5 doses of levo were non-inferior to 20 doses of cipro. Levo and cipro eradicated blood pathogens in all subjects who had a 2nd blood culture.

O143 Baseline characteristics of patients, with or without MRSA, in two double-blind, randomised, multinational, Phase 3 studies comparing telavancin with vancomycin for the treatment of complicated skin and skin structure infections

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Objectives: Telavancin is a novel, rapidly bactericidal lipoglycopeptide with concentration-dependent killing activity against clinically important Gram-positive pathogens. It has a unique multifunctional mechanism of action that includes inhibiting bacterial cell wall synthesis and disrupting the functional integrity of the bacterial plasma membrane. Two identically designed, double-blind, randomised, multinational Phase 3 studies, ATLAS 1 and ATLAS 2, have compared the efficacy and safety of telavancin with vancomycin in adults with a complicated skin and skin structure infection (cSSSI).
Methods: The ATLAS studies recruited men and women, who were 18 years of age or over. All patients had a cSSSI (major abscess, infected burn, deep/extensive cellulitis, infected ulcer or wound infection) requiring ⩾7 days of IV antibiotic therapy. Here we report the baseline demographic and clinical characteristics of study participants by MRSA status.
Results: Of the 1867 patients treated in the two studies, 38.5% (n = 719) had an infection caused by MRSA (37.7% [350/928] of telavancin-treated patients and 39.3% [369/939] of vancomycin-treated patients). Approximately two-thirds of patients were from the USA; the remainder were recruited from Canada, Australia, South Africa, Russia and various other countries across Europe, Asia and South America. Patients with infections due to MRSA tended to be younger (<65 years of age) and a greater proportion were of Black race compared with patients with infections not due to MRSA (non-MRSA; Table). MRSA was most commonly associated with major abscesses, whereas non-MRSA was most commonly associated with deep/extensive cellulitis. Regardless of MRSA status, cSSSI occurred more frequently on the lower extremities.

Table. Patient demographic and clinical characteristics

	MRSA[a] (n = 719)	Non-MRSA (n = 1 148)
Age, years (mean)	44.6	51.4
Age <65 years, n (%)	632 (88)	882 (77)
Men, n (%)	421 (59)	655 (57)
Race[b], n (%)		
Caucasian	536 (75)	911 (79)
Black	149 (21)	111 (10)
Hispanic/Latino	140 (19)	247 (22)
Diabetes, n (%)	149 (21)	315 (27)
Type of cSSSI[c], n (%)		
Major abscess	423 (59)	367 (32)
Wound infection	115 (16)	153 (13)
Deep/extensive cellulitis	153 (21)	538 (47)
Infected ulcer	20 (3)	73 (6)
Infected burn	8 (1)	17 (1)
Site of infection, n (%)		
Head/neck	58 (8)	71 (6)
Front torso	111 (15)	149 (13)
Back torso	108 (15)	99 (9)
Upper extremities	145 (20)	179 (16)
Lower extremities	297 (41)	650 (57)

[a]MRSA, methicillin-resistant *Staphylococcus aureus*.
[b]Patients could indicate more than one racial category.
[b]cSSSI, complicated skin and skin structure infection.

Conclusion: Together, ATLAS 1 and 2 represent the largest prospective, double-blind studies ever conducted in patients with cSSSIs. The MRSA cohort was also the largest ever enrolled in a registrational programme. MRSA infections appeared to be more common in patients who were younger, of Black racial origin, and in those with a major abscess compared with patients with infections not due to MRSA.

O144 Efficacy and safety of aminoglycoside monotherapy: systematic review and meta-analysis of randomised controlled trials

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Objectives: The use of aminoglycosides has decreased due to doubts regarding lack of efficacy and adverse effects. Yet, the prevalence of microbial resistance to aminoglycosides has remained low. This study sought to compare the efficacy and adverse effects of aminoglycosides as a single antibiotic to other antibiotics for the treatment of patients with infection.
Methods: We searched for randomised trials comparing the efficacy of aminoglycoside antibiotic treatment to non-aminoglycoside antibiotic in patients with infection in the Cochrane Library, MEDLINE, EMBASE, LILACS, databases of ongoing trials and conference proceedings. Two reviewers assessed trial eligibility, quality and extract data. Pooled relative risks (RR) with 95% confidence intervals (CI) were calculated for dichotomous data.

Results: The search yielded 37 trials. 27 trials assessed patients with urinary tract infection, 5 trials assessed patients with other source of infection and 5 did not define the source of infection. Aminoglycosides were equally effective as comparator in analysis of mortality (RR 1.26, 95% CI: 0.68, 2.31, n = 458) and treatment failure (RR 1.10, 95% CI: 0.96, 1.27, n = 1908), but were associated with significant more bacteriological failure (RR 1.39, 95% CI: 1.14, 1.68, n = 1242). Subgroup analyses according to quality of trial, type of antibiotics, source of infection and, rate of clinical sepsis did not alter the outcomes. Less adverse effects in total but more nephrotoxic effects were observed in patients treated with aminoglycosides.
Conclusions: The present data supports the use of aminoglycosides as treatment for urinary tract infection but not as single treatment for patients with infections other than the urinary tract.

Molecular typing

O145 Microarray technology reveals genomic diversity and phylogeny in *Mycobacterium ulcerans*

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Background: *Mycobacterium ulcerans* is the etiologic agent of Buruli ulcer, the third most common mycobacterial disease occurring in more than 30 countries and characterised by devastating chronic necrotising skin ulcers. Elucidation of the transmission and epidemiology of *M. ulcerans* is hampered by a remarkable lack of genetic diversity, and no molecular genotyping method is available that has sufficient high resolution for micro-epidemiological studies.
Methods: We conducted comparative genomic hybridisation of 30 *M. ulcerans* strains of world-wide origin with a newly developed plasmid-based microarray technology, suitable for organisms lacking genome sequence information. Resulting genomic differences were analysed by PCR and sequencing and subjected to in silico sequence comparison with *Mycobacterium marinum*.
Results: The microarray based comparative genomic analysis revealed first extensive large insertional/deletional sequence polymorphisms among the clinical *M. ulcerans* isolates, depicting progressing genome shrinkage. Categorisation of the deleted genes revealed biological functions possibly no longer required for the pathogen developing from the environmental mycobacterium, *M. marinum*. In depth analysis showed evidence that the Asian and Southern American strains are closer to the progenitor than isolates from African and Australian countries, giving first hints towards the existence of two major lineages of the pathogen *M. ulcerans* with different evolutionary histories in their genome arrangement.
Conclusions: The comparative analysis of the genomes of *M. ulcerans* isolates suggests that this emerging pathogen is adapting to a more stable environment, probably to a mammalian host including man. The significant genomic diversity revealed by analysis with a prototype microarray suggests that a whole genome microarray, currently under development, may lead to a genomic fingerprinting method urgently needed for micro-epidemiological studies and aiming to characterise transmission pathways and environmental reservoirs of *M. ulcerans*.

O146 Genotypic and phenotypic diversity of clinical *Bordetella pertussis* isolates in the UK from 1920 to 2005

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Objectives: To measure the genetic diversity of the population of *B. pertussis* clinical isolates at different times in UK anti-pertussis vaccination history using multi-locus VNTR analysis (MLVA) and to further characterise isolates using ptxA and prnA genotyping and fimbrial serotyping.
Methods: More than 600 isolates collected between 1920 and 1995 were divided into groups around important dates in UK vaccination history and typed using a 6-allele MLVA scheme. Genetic diversity was calculated from the resulting MLVA types using the method of Hunter and Gaston. ptxA and prnA genotyping and fimbrial serotyping were also performed.
Results: MLVA revealed that the genetic diversity of UK *B. pertussis* isolates was greatest during the period before mass vaccination with whole-cell vaccines was introduced in 1957 [diversity index (DI) = 0.88 (n = 69)]. Between 1963–1967, when vaccine coverage was ~80%, diversity fell [DI = 0.74 (n = 14)] and MLVA-29, previously a minor type, became dominant (50% of isolates).
Between 1977–1986, when vaccine coverage fell to a low of 31% and epidemics occurred in the UK, diversity increased to a level almost as high as before vaccination [DI = 0.84 (n = 157)]. Interestingly, during this period MLVA-29 was still the most common type, but many new MLVA types, the prnA(2) and prnA(3) genotypes and the serotype 1,3 emerged. MLVA-27 first appeared in 1983.
In 1998–2001, several years after vaccine coverage had risen to >95%, DI had fallen again to 0.66 (n = 108) and MLVA-27 was co-dominant (43%) with MLVA-70 (41%). During the period 2002–2005, when vaccine coverage remained high and a fourth dose was added to the schedule, DI declined further and MLVA-27 increased its dominance. In 2005, DI = 0.46 (n = 109) and 73% of isolates was MLVA-27.
Conclusion: MLVA reveals much greater genetic diversity in the UK *B. pertussis* population than previously shown using prnA and ptxA genotyping and fimbrial serotyping. Genetic diversity fell during periods of high vaccine coverage, but increased when vaccination dropped as the result of a health scare in the late 1970s. During this window of high diversity, many new MLVA types emerged and new prnA, ptxA and fimbrial serotypes appeared. In recent years, under high vaccine coverage, diversity has fallen and a single clone "MLVA-27 prnA(2) ptxA(1) serotype 1,3" has come to dominate the UK population.

O147 Precise identification and phylogenetic relationships of major multi-resistant clones of *Acinetobacter baumannii* by multilocus sequence typing

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Multidrug resistant (MDR) *Acinetobacter baumannii* strains that infect hospitalised patients are among the most dreadful nosocomial bacteria in European and US hospitals. Evidence from banding pattern typing methods (AFLP and ribotyping) suggested that three clones are responsible for a majority of hospital outbreaks caused by MDR strains in European hospitals (Dijkshoorn et al., 1996; Van Dessel et al., 2004). These three pan-European clones were recently shown to be responsible for more than 60% of MDR *A. baumannii* infections in a US hospital (Hujer et al., 2006). Precise identification of strains belonging to clones I to III is therefore crucial for infection control and patient management. However, banding-pattern methods are difficult to standardise and provide limited information on phylogenetic relationships among strains. Using MLST, i.e. sequencing of internal portions of seven housekeeping genes (total 2,976 nt), we analysed a set of 155 strains of *A. baumannii*, including 80 strains belonging to clones I, II and III and 75 other strains representing the breadth of known AFLP diversity. A total of 59 distinct sequence types (STs) were found. Phylogenetic analysis based on allelic profiles revealed that strains from clones I, II and III fell into three unrelated clonal complexes, each made of a central, predominant genotype and a few (one to four) single locus variants. The only exception was one double-locus variant strain of clone III. Interestingly, the 75 strains from the AFLP diversity set had at least three distinct loci when compared to members of the MDR clones. We conclude that each of the three major clones can readily be demarcated from other *A. baumannii* members, and that molecular diagnostics could be developed to identify them rapidly. Analysis of the recombination/mutation rate shows that nucleotides do not change by recombination more frequently than by mutation, showing that recombination is unlikely to disrupt the clonal frame of the clones, which can therefore be expected to be stable over very long periods of time.

O148 Correspondence between typing methods results: a web-based quantitative analysis tool

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Objectives: Molecular epidemiology studies of clinically relevant microorganisms frequently focus on a comparison between the assigned types of different typing methods. Therefore, there is a critical need to understand the correspondence between types produced by different methods. This may be useful not only for the comparison of the genetic backgrounds of the particular set of isolates under study but also to produce a broader view of how the results of the different typing methods are related. To achieve this goal, a framework of measures for the quantitative comparison of typing methods results was developed (Carriço et al., Illustration of a common framework for relating multiple typing methods by application to macrolide-resistant *Streptococcus pyogenes*, J. Clin. Microbiol. 2006) and is now implemented in a free-access web-based interface, on which users can analyse their data anonymously and retrieve the results.
Methods: To facilitate the use of the framework developed, a web-based interface was implemented to provide researchers with a user-friendly interface to compare their typing methods results. On the website, we also provide a set of Bionumerics^{TM} scripts to allow the users to implement the proposed analysis on their Bionumerics databases while offline.
Results: The web-based interface and the Bionumerics scripts allow for the calculation of several measures: Simpson's index of diversity with confidence intervals for accessing the diversity of typing results for different methods; Adjusted Rand and Wallace coefficients for bidirectional and unidirectional (respectively) comparison between the results of two typing methods results. Based on these measures the users can quantitatively evaluate the discriminatory power of the typing methodologies used and their concordance.
Conclusion: The web-based interface and the Bionumerics scripts provide users with the ability for a quantitative assessment of correspondence between typing methods results. This can be used to evaluate the predictive power of a typing methodology when compared to another, which can guide the user in the choice of a 'gold standard' for clone definition. Furthermore, as new microbial typing methods are proposed, this methodology allows for their comparison in terms of type assignments with established methodologies.

O149 Multi-locus sequence typing for surveillance of *Campylobacter*

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Despite several case control studies and enhanced surveillance of both human cases and animal environmental isolates, there is still much unknown about the epidemiology of *Campylobacter* infection. We describe a multi-agency project evaluating the utility of sequence typing of human infection isolates to inform surveillance and distinguish environmental sources of human infection.
Multi-locus sequence typing (MLST) developed for *C. jejuni* and *C. coli* was applied to all human case isolates from four local authorities in North West England, two largely rural and two largely urban, over a continuous 3-year period. Isolates from drinking water supplies and recreational surface waters were also sequence typed.
We describe here an analysis of the three year study data and steps taken to adapt the methodology for use in routine surveillance settings. The study has demonstrated several interesting correlations between specific sequence types and a number of explanatory variables for disease (eg month of infection, locality of residence, and travel abroad). Other studies provide increasing evidence of host-association for specific MLST types of campylobacter and these findings are related to the three year dataset. In addition, many new types have been described in surface waters from this project and there is evidence for a distinct water-borne population that has not so far been described in human infection.

Tick-borne diseases

S154 Rickettsioses

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In recent years, the use of cell culture and molecular biology deeply changed our knowledge on rickettsiae. As a matter of fact before 1990, 7 pathogenic rickettsial species were identified (*Rickettsia prowazekii*, *R. typhi*, *R. conorii*, *R. rickettsii*, *R. sibirica*, *R. australis*, *R. acari*) and since 1991, 10 new species were discovered (*R. japonica*, *R. honei*, *R. africae*, *R. slovaca*, *R. parkeri*, *R. helvetica*, *R. aechlimanii*, *R. massiliae*, *R. heilongjanghensis* transmitted by ticks and *R. felis* transmitted by fleas) and 2 new species (*R. conorii caspia*, *R. sibirica mongolotimonae*).
New rickettsial diseases were found under 3 main conditions:
– In place where none was identified, typical rickettsial diseases (including fever and a rash) were found (Japan, China).
– In some place typical rickettsioses could be caused by different organisms. In such cases, the new *Rickettsia* was misdiagnosed with a previously identified bacterium (such as *R. massiliae* with *R. conorii*).
– In some cases atypical clinical findings were found (no rash, no fever) to be caused by Rickettsial organisms such as *R. slovaca* or *R. helvetica*.

These findings challenge the old dogma postulating that of one tick borne rickettsiosis was prevalent in one geographic area. For many years for example, *R. rickettsii*, the agent of Rocky Mountain Spotted Fever, was considered the only spotted fever group rickettsia in the USA and the only tick-transmitted rickettsiosis in America. *R. felis*, a flea-transmitted spotted fever, and *R. parkeri*, a tick-transmitted spotted fever, have been shown since to infect human beings in the USA and in Urugay. Moreover, *R. africae* has been found in patients in West Indies.
Many *Rickettsia* have been identified in ticks but have not been currently found in patients. These *Rickettsiae* should be considered potential pathogens. These new findings should stimulate investigations to identify new rickettsial diseases. Patients with atypical rash or fever after arthropod bite should be targeted. Skin biopsies are the preferred samples in this purpose. Molecular tests used for this purpose will be proposed.

Can pharmacokinetic–pharmacodynamic parameters drive dosing regimens that are less vulnerable to resistance?

S156 Can pharmacokinetic–pharmacodynamic parameters drive dosing regimens that are less vulnerable to resistance? Pro

A. Novelli (Florence, IT)

In recent years, the rules for selection of antimicrobial agents have been undergoing critical revision in terms of optimum dosing for control of infectious diseases, with the goal of potentiating treatment efficacy and reducing the risk of selecting multidrug resistant pathogens. The most important criterion for rational choice of an antimicrobial agent is defined by its pharmacodynamic (PD) characteristics, and thus its antimicrobial activity which can be summarised as the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The second criterion for selecting an antibiotic is due to its pharmacokinetic (PK) characteristics, since it has been demonstrated that antibiotic concentrations at the infection site influence the intensity and duration of the effect, and together with the PD parameters, provide a general inter-relationship and contribute to defining the potential clinical efficacy of a drug. Pharmacodynamics have been upgraded in the last 10 years with in vitro and animal models which have helped to identify fairly precise correlations with therapeutic efficacy of antibiotics. Moreover, the principles that link concentrations

of antibiotics in humans and their effects have been outlined in order to determine the optimal dosing interval. Antimicrobial drugs with a concentration-dependent activity, i.e. the newer quinolones and aminoglycosides, should be administered as a single daily dose to maximise the peak serum concentration/MIC ratio. AUC/MIC ratio is also an important parameter for PK/PD correlations. Existing data on fluoroquinolones suggest that a ratio of 100–125 correlates with high bacterial eradication and optimal clinical outcome in infections due to Gram-negative pathogens, while a ratio of 50 is associated with a high probability of eradication of *S. pneumoniae* strains. For this group of antibiotics the new concept of the so called mutant prevention concentration (MPC) and mutant selection window (MSW) may be helpful in restricting the enrichment of mutant subpopulations and consequently, at least partially, in controlling the spread of resistance. Beta-lactams, having time-dependent efficacy, usually do not have great post antibiotic effects (PAEs), and the parameter which seems to better correlate PD with PK is the time duration with concentrations higher than the MIC (T > MIC). These antibiotics need to be given with short dosing intervals or even by continuous infusion to maintain plasma levels exceeding the MIC for a sufficiently long period.

In summary, we can theoretically optimise the dosage regimen of antibiotics (dose, administration route and interval between doses) in clinical practice by correlating the PK and PD pertaining to each antibiotic class, based on experimental animal studies and these PK/PD parameters may contribute to the containment of resistance for all drug classes and especially the most important ones used in serious infections in intensive care patients.

Antibiotic resistance in developing countries: a Pandora's Box

S159 Surveillance of antimicrobial resistance in developing countries: methods and strategies

A. Bartoloni (Florence, IT)

Antibiotics are the most commonly purchased class of drugs in low-resource countries, where the infectious diseases are extremely frequent and the bacterial infections are the major cause of death, especially in childhood. The phenomenon of microbial drug resistance, which represents a global public health problem, is particularly serious in these countries, as resistance rates are even higher than in industrialised countries and the therapeutic options are often unavailable or too expensive. Surveillance of antibiotic susceptibility is a key element to provide updated information on the magnitude and trends in resistance, and to plan and monitor intervention strategies aimed at preserving the therapeutic efficacy of antibiotics. In low-resource countries, effective surveillance programmes are difficult to implement for a number of reasons, including scarce financial resources, lack of laboratory facilities and, where laboratories do exist, lack of quality control, reliable reagents and adequate supervision. In these settings, the development of reliable and low-cost alternative methods could facilitate the implementation of large-scale surveillance. There is an increasing agreement about the importance of extending the surveillance of antibiotic resistance to the commensal microbiota of humans and animals. This bacterial population, although not being a specific target, is continuously exposed to the selective pressure generated by antimicrobial chemotherapy and may become a potential reservoir of resistant strains that can cause infections, and of resistance determinants that can be transferred to pathogenic bacteria. Therefore, surveillance of antibiotic-resistant bacteria carried by healthy individuals is considered an indicator of the spread of antibiotic resistance that could also be useful to predict the emergence of resistance in pathogenic bacteria. In this perspective, resistance patterns of some members of the commensal microbiota, such as the faecal *Escherichia coli*, have been evaluated in various epidemiological settings. For this purpose, different microbiological approaches have been implemented and evaluated as useful tools to conduct large scale resistance surveillance studies and to monitor resistance control programmes in a cost-effective manner.

Does detection of extended-spectrum β-lactamases matter?

S160 Does detection of extended-spectrum β-lactamases matter? The No case

J. Turnidge (North Adelaide, AU)

The emergence of extended-spectrum β-lactamases (ESBLs) in Enterobacteriaceae in the 1980s marked an important turning point in routine laboratory diagnostics. Soon after their discovery, phenotypic methods were developed for their detection and confirmation. Confirmation depended largely on the ability of clavulanate to inhibit the main culprit enzymes at the time, the TEM and SHV variants. Later this technique proved reliable for CTX-M type enzymes. Many susceptibility testing methods recommended the use of ESBL screening and confirmation tests on a routine basis, including CLSI, BSAC, CA-SFM, and SRGA. However, a number of issues have emerged with routine use over the years:

1. The problem of defining an adequate number of substrates to ensure sufficiently sensitive screening. Ideally, one should include a minimum of 4, namely cefpodoxime, ceftazidime, ceftriaxone or cefotaxime, and aztreonam, and at concentrations that often differ from those used for susceptibility breakpoints.
2. The lack of reliable phenotypic methods to detect ESBLs in species with inducible AmpC β-lactamases. Some of these species have been shown to be important reservoirs for ESBLs, and resistance to extended-spectrum cephalosporins cannot solely attributed to stable de-repression of AmpC.
3. The failure of current methods to provide advice on the interpretation of a positive screening test but a negative confirmation test, especially if the isolates are "susceptible" to extended-spectrum cephalosporins using method-recommended breakpoints. Such strains have been shown to harbour OXA enzymes, inhibitor-resistant TEM enzymes, or particularly plasmid-borne AmpC enzymes with significant frequency. Thus there is no current phenotypic or genotypic test that can be practically and effectively applied in the routine laboratory with sufficient sensitivity to detect the emerging range of transmissible enzymes.

However, we can rely to a great extent on the selection of or change to appropriate susceptibility breakpoints. A range of recent studies has suggested that failures of treatment with extended-spectrum cephalosporins are likely when strains of Enterobacteriaceae have MICs elevated above the wild-type. Further, application of pharmacokinetic/pharmacodynamic (PK/PD) principles to the most widely recommended dosing schedules of cephalosporins suggest that the susceptibility breakpoints recommended by many methods are too high, and should to lowered to values that fortunately coincide wild-type cut-off values. Hence, lowering of susceptibility breakpoints for extended-spectrum cephalosporins to those defined by PK/PD will indicate the presence of an ESBL or plasmid-borne AmpC enzyme with sufficiently high likelihood to allow laboratories to report both resistance to these agents and provide advice about appropriate infection control procedures.

Antibiotic usage

O164 A planned dramatic drop in trimethoprim consumption in a 180,000 population did not result in a related decrease in trimethoprim resistance in *Escherichia coli*

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Objectives: Since antibiotic resistance often is associated with a biological fitness cost for bacteria it is assumed that a reduction of antibiotic use is followed by a reduction in resistance rates. Over the last 10 years trimethoprim (TRI) resistance in *Escherichia coli* has increased in Sweden. In Kronoberg county the TRI resistance has increased from

7% (1990) to 11% (2004). So far no prospective intervention in the community has been carried out to investigate whether a substantial decrease of the use of a single antibiotic will result in a corresponding decrease in antibiotic resistance.
Method and Material: Physicians (n = 564) in county Kronoberg (approx. 180,000 population), Sweden, were convinced, by personal visits and mail, to cease their use of TRI and cotrimoxazole during two years starting October 1st 2004. Monthly sales data for oral antibiotics were retrieved from the National Corporation of Swedish Pharmacies and made known to the physicians. All *E. coli* isolated from urinary tract specimens at the Dept of Clin Microb, Växjö, were included in the analysis. The susceptibility testing methodology was stable since 1990 and the baseline consisted of quantitative data from 1990–2004. In order to study the diversity among the isolates 2900 *E. coli* from the period preceding and 1200 isolates from the end of the intervention were phenotyped using the PhenePlateTM system.
Results: An immediate and sustained decrease of 85% in total use of TRI was achieved. TRI use was in most cases replaced by mecillinam (MEC), nitrofurantoin (NIT) and ciprofloxacin. The total decrease in antibiotics used for UTI treatment was 4%. Resistance to TRI did not decrease (10% and 12% in 2005 and 2006, respectively). Resistance to NIT and MEC did not increase despite the substantial increase in the use of these drugs, 31% and 69%, respectively. Resistance to fluoroquinolones (FQ) increased from 4% to 10% between 2000 and 2006. The phenotypic diversity index was 0.954 in the period prior to the intervention, 0.964 during the first 4 months and 0.949 during the last 4 months of the intervention.
Conclusion: A substantial and sustained decrease in the use of TRI in a 180,000 population did not result in any drastic changes in resistance rates. Whether the increase in FQ-resistance was related to the increase in use remains to be evaluated. The diversity index, based on *E. coli* phenotypes, was stable indicating that the intervention did not favour any certain clone to expand in the community.

O165 Sustained reduction of antibiotic use and low bacterial resistance. A ten-year follow-up of the Swedish Strama programme

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Objectives: Increasing use of antibiotics and spread of resistant pneumococcal clones in the beginning of the 90ies alarmed the medical profession and medical authorities in Sweden. A coordinated effort to prevent further spread and preserve the effectiveness of antimicrobial agents was therefore initiated
Methods: Strama (The Swedish strategic programme for the rational use of antimicrobial agents and surveillance of resistance) was initiated in 1994. The Strama network includes a broad representation of medical disciplines, professional organisations and relevant authorities. The programme includes surveillance of antibiotic use and resistance, implementation of rational use of antibiotics and development of new knowledge. The main goal is to preserve the effectiveness of antimicrobial agents.
Results: Yearly validated data on antibiotic use and resistance were made publicly available on a website. Multidisciplinary Strama-groups were formed in each county to disseminate knowledge and implement rational antibiotic use. Studies were performed on indications for antibiotic use in out-patient care, hospital care and nursing homes.
Between 1995 and 2004 antibiotic use for out-patients decreased from 15.7 to 12.6 DDD/TID and from 536 to 410 prescriptions per 1000 inhabitants and year. The reduction was most prominent for children 5–14 years old (52%) and for macrolides (65%). During the period, the number of hospital admissions for acute mastoiditis, rhinosinusitis and quinsy was stable or declining. Although the epidemic spread in southern Sweden of penicillin-resistant *S. pneumoniae* was curbed, the national frequency increased from 4% to 6%. A hospital outbreak of MRSA could be terminated using aggressive infection-control measures. Resistance remained low in most other bacterial species during the period.
Conclusions: This multidisciplinary, coordinated programme has contributed to the reduction of antibiotic use without measurable negative consequences. Despite this, antibiotic resistance in several bacterial species is slowly increasing which calls for continued sustained efforts to preserve effectiveness of available antibiotics. Such efforts will include interventions to further improve antibiotic use and to improve compliance to basic hygiene precautions.

O166 ESAC II Hospital Care Subproject 2005–2007: patterns of antibiotic use in relation to diagnoses in 19 European hospitals in 2006, Point Prevalence Study

M. Erntell, F. Ansari, H. Goossens, P. Davey for the ESAC II HC Subproject Working Group

Objectives: 19 European hospitals performed a descriptive pilot Point Prevalence Study (PPS) of antimicrobial use in relation to diagnoses in European hospitals.
Method: The protocol was designed to present demographic data as well as the amounts and indications for antimicrobial agents against bacteria. Treatments were recorded in relation to diagnoses, prophylactic use, community acquired (CAI) and hospital acquired infection (HAI). 19 pre-defined diagnosis groups were used. The previously presented STRAMA protocol and web-based reporting system was used.
Results: 19 hospitals participated in the study. 3,398 patients treated with antimicrobial agents were included out of 11,224 admitted. 30% of the patients were treated with antimicrobials. 3,554 treatments were recorded. 377 (10.6%) were given to children (<17 years) and 47.6% to women. The indication for treatment was CAI in 15%, HAI in 9.2% and prophylaxis in 7.6%. For adults cultures were taken before parenteral treatment in 57% and oral treatment in 51%. The most commonly used antimicrobials for adults, in DDD, in treatment and prophylaxis were penicillins with betalactamase inhibitors (23%, 0–75, and 26%, 0–66), cephalosporins (14%, 4–38, and 30%, 0–60), fluoroquinolones (14%, 8–31, and 11%, 0–52). The total amount of antimicrobials used for adults was 52 DDD/100 admitted patients (33–88). Two diagnosis groups were predominating; pneumonia (19% of all therapies) and skin and soft tissue infections (13%). Analysis of antibiotic usage in different countries shows countries having mainly penicillins with betalactamase inhibitor or cephalosporins as the predominating drug. Seven countries showed a more varied use of drugs. The patterns of treatment and prophylactic usage were similar in countries with heavy use of one ATC-class. Length of peri-operative prophylaxis was dominated by >1 day in all surgical specialities; in general surgery 59%, in orthopaedic surgery 52%, in urology 76% and in ENT 89%. One-dose peri-operative prophylaxis ranged from 2% to 27%. One-day prophylaxis was 34% in orthopaedic surgery.
Conclusions: The study describes wide differences (three fold) in consumption between European hospitals. Peri-operative prophylaxis is too long in all surgical specialities. There is limited variation in the use of different antibiotic groups in most hospitals – a risk factor for emergence of antibiotic resistance. Our web-based PPS provides a tool for quality assessment of antibiotic prescribing in European hospitals.

O167 Attitudes, beliefs and knowledge concerning antibiotic use and self-medication: a comparative European study

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Introduction: Although the relevance of cultural factors for antibiotic use is widely recognized, few studies exist in Europe. We compared beliefs, attitudes and knowledge regarding antibiotic use and self-medication between countries.

Methods: Face to face structured interviews were conducted in 11 countries (Austria, The Netherlands, Sweden, UK, Belgium, Italy, Malta, Israel, Czech Republic, Lithuania and Croatia). The study population included respondents of our previous survey who agreed to be interviewed. We aimed to recruit at least 100 respondents in each country, including a group of users of self-medication and non-users. Scales were grouped based on theoretical concepts and confirmed by factor analysis. The four scales were: attitudes towards appropriateness of self-medication with antibiotics for bronchitis, beliefs about antibiotics for minor ailments, attitudes towards situational use of antibiotics and knowledge about antibiotics and viruses or bacteria. Knowledge of antibiotic resistance was measured by an open-ended question. Analysis: To deal with the possible confounding effect of both use of self-medication and education, we performed stratified analyses, i.e., we studied the differences between countries separately for users and non-users of self-medication, and for respondents with high and low education. The differences between countries were considered relevant when regression coefficients were significant in all stratum-specific analyses.
Results: In total, 1101 respondents participated. Respondents from the UK, Malta, Italy, Czech Republic, Croatia, Israel and Lithuania had significantly less appropriate attitudes, beliefs or knowledge for at least one of the dimensions. The Dutch, Austrian and Belgian respondents did not differ from Swedish for any of the dimensions.
Conclusions: Our study showed clear cultural differences in levels of public attitudes, beliefs and knowledge concerning antibiotic use, self-medication and antibiotic resistance in 11 European countries. The levels of misconceptions contributing to inappropriate use were the highest in southern and eastern countries, indicating a strong need for public education campaigns in these countries. Awareness about antibiotic resistance was the lowest in countries reporting high prevalence of resistance.

O168 Optimising use of ciprofloxacin in a large teaching hospital: sustained effect of a prospective intervention study

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Objective: Antimicrobial resistance to ciprofloxacin, a valuable second-line antibiotic, is increasing. To limit this increase the appropriate use of ciprofloxacin should be encouraged. The objective of this study was to reduce the number of inappropriate prescriptions and improve the quality of ciprofloxacin prescriptions by way of educational intervention.
Methods: Five units (197 beds) of the departments of Internal Medicine, Gastro-Enterology, Surgery, Urology and Pulmonary Diseases, selected because of a high rate of ciprofloxacin prescription, participated in a prospective intervention study. The study comprised three periods of three months: 2 observation periods (phase 1 and 3) and an intervention period (phase 2). A follow up of 3 months was done 9 months after start of intervention. During the two observation periods all ciprofloxacin prescriptions were registered and the quality of each ciprofloxacin prescription was evaluated in a standardised manner by two experts in infectious diseases independently. During the intervention period physicians prescribing ciprofloxacin were interviewed by a medical microbiologist, and educational presentations were given to physicians of the participating units.
Results: During phase 1 491 prescriptions per 1000 admissions of ciprofloxacin were written, declining to 184 prescriptions per 1000 admissions in phase 3, a reduction of 62.5%. The greatest reduction was observed in units of the Departments of Surgery and Urology (83.9% and 75.6% respectively), mainly due to a reduction of erratic prophylactic use. Unjustified prescriptions (no use of antibiotics indicated) decreased with 25.9%. Inappropriate prescriptions (wrong choice of antibiotic or duration of prescription) declined from 69.5% to 57.7%, mainly due to the decrease of ciprofloxacin courses of too long duration. Appropriate prescriptions increased with 33.5%. Nine months after intervention 136 prescriptions per 1000 admissions of ciprofloxacin were prescribed, a total reduction of 72.3%.
Conclusion: Intervention by direct consultation of a medical microbiologist and educational presentations led to significant reduction of the use of ciprofloxacin and improvement of the quality of ciprofloxacin prescription. Nine months after the intervention the use of ciprofloxacin had declined even further, indicating a sustained effect of the intervention measures.

Rare fungal infections

O169 High-dose amphotericin B with flucytosine for the treatment of cryptococcal meningitis in HIV: a randomised study

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Objectives: To determine the early fungicidal activity (EFA) and toxicity of high dose amphotericin B (AmB, 1 mg/kg/d) plus flucytosine (5FC) compared with standard dose amphotericin B (0.7 mg/kg/d) plus flucytosine for the treatment of HIV-associated cryptococcal meningitis.
Methods: 64 HIV positive antiretroviral therapy naive patients, presenting with a first episode of cryptococcal meningitis in Cape Town, South Africa from May 2005 to June 2006, were randomised to receive either amphotericin B 0.7 mg/kg/d plus flucytosine 25 mg/kg qds (Group 1) or amphotericin B 1 mg/kg/d plus flucytosine 25 mg/kg qds (Group 2) for 2 weeks, followed by oral fluconazole. EFA was evaluated by the rate of reduction in CSF cryptococcal colony-forming units (CFU) determined from serial quantitative CSF cultures on days 1, 3, 7, and 14 of treatment. Follow-up was 10 weeks.
Results: Median CD4 count was 38×10^6/L. 13% of patients had abnormal mental status. Mean (SD) early fungicidal activity was –0.56 (0.24) log CFU/mL CSF/d for AmB at 1 mg/kg/d plus 5FC and –0.45 (0.16) CFU/mL CSF/d for AmB at 0.7 mg/kg/d plus 5FC. As in prior studies, baseline count was associated with rate of clearance. In a linear regression model including treatment group and baseline count, EFA was significantly greater for AmB at 1 mg/kg/d plus 5FC compared with AmB at 0.7 mg/kg/d plus 5FC (difference 0.12 log CFU/d [95% CI: 0.02–0.23], p = 0.02). There was a trend towards a greater rise in creatinine in Group 2 versus Group 1: final creatinine increased by 88% vs 52% from baseline (p = 0.08), which was reversible on stopping study drug treatment. Mortality was 23% (15/64) at 10 weeks with no difference between the two groups.
Conclusions: Amphotericin B at 1mg/kg/d with flucytosine is more rapidly fungicidal in the treatment of HIV-associated cryptococcal meningitis compared to standard dose amphotericin B. Increasing the dose of amphotericin B did not result in a clinically significant increase in nephrotoxicity.

O170 Clinical and epidemiological aspects of locally-acquired *Cryptococcus gattii* human infections, an emerging fungal pathogen in British Columbia, Canada

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Cryptococcus gattii emerged in the province of British Columbia (BC), Canada in 1999. This study was undertaken to describe the epidemiological and clinical features of locally-acquired *C. gattii* infections in humans in BC.
Cases diagnosed by culture and confirmed by restriction fragment length polymorphism as *C. gattii* and HIV negative cases diagnosed by serology and histopathology with no exposure to international endemic areas were included. Cases reported to provincial authorities between 1999 and 2006 were interviewed to determine clinical presentation and epidemiological characteristics. Frequencies were calculated based on denominators available using SPSS® v14.0.
As of November 16 2006, 162 cases of locally-acquired cases of *C. gattii* infection occurred in BC with an annual provincial average of 6.3 cases/million population. Mean age was 59 years (2–92 years) and 56% were male. 16 cases (10%) were asymptomatic.

142 (88%) had pulmonary, 28 (17%) had central nervous system and 2 (1%) had dermatological findings. Most common symptoms included cough (46%), shortness of breath (44%), fever and night sweats (both 35%). Chest X-rays revealed nodules in 65 cases (66%) and infiltrates in 14 cases (14%). 19 cases (30%) were on oral steroids in the year prior to diagnosis and 50 (49%) were current smokers. Only 5 (4%) were HIV positive, 3 (3%) were organ transplant recipients, 30 (31%) had a chronic lung disease and 26 (26%) had a history of cancer. 103 cases (64%) were culture-confirmed as *C. gattii* and 81 isolates (79%) were genotyped as VGIIa. 4 cases (3%) died due to their *C. gattii* infection. Most cases were treated with fluconazole and amphotericin B for several months. Most cases were exposed on Vancouver Island but the range of the fungus has spread over the years and 4 recent cases were exposed on the BC mainland.
Clinicians should be aware that *C. gattii* can be acquired in Canada and that the epidemiological and clinical characteristics of this disease differ from *C. neoformans*.

O171 A comparative study of treatment outcome in cryptococcal meningitis in HIV-infected patients during period before versus after implementation of treatment guidelines for management of increased intracranial pressure

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Background: Cryptococcal meningitis is a common opportunistic infections in HIV patients in Thailand, despite the expanded resources for HIV treatment. Most patients had poor treatment outcome due to poor control of severely increased intracranial pressure (ICP). This complication may arise before, during or after the antifungal therapy. Our study aimed to compare the treatment outcome in these patients before and after implementation of the strategies particularly regarding the management of severely elevated ICP.
Methods: We retrospectively reviewed the clinical data, treatment and outcome of all adult HIV patients with cryptococcal meningitis who were admitted at our tertiary care centre from 2002–2003 and 2004–2005. The inclusion criteria included all patients who had meningitis and either positive cerebrospinal fluid (CSF) cultures for *Cryptococcus neoformans* or cryptococcal antigen.
Results: There were 107 and 165 patients during the period before and after the implementation of the guidelines. 250 (92%) had positive cultures. No different baseline characteristics between the two groups were observed. 52 (9.5%) patients had recurrent cryptococcal meningitis despite fluconazole prophylaxis. The mean age was of 37.4 years. The median baseline CD4 cell counts was 55 cells/mm^3. 39 (14%) patients had received antiretroviral therapy. Most of the patients had very high ICP. The mean and median CSF opening pressure (OP) were 34 and 38 cmH_2O. 222 (85%) and 24 (14%) patients had OP of >20 and >40 cmH_2O, respectively. CSF sterilisation could be achieved in 182 (70%) patients by amphotericin B treatment with a mean duration of 18 days. Before adoption of guidelines, high ICP was treated by the judgement made by individual primary care physicians. Strategies for management of elevated ICP included 111 (42%) patients with continuous once or twice daily lumbar punctures (LPs), 11 (4%) with lumboperitoneal shunts, and 14 (5%) with ventriculoperitoneal shunts. 2 patients (0.7%) had emergency shunt surgery (impending visual acuity loss or hearing loss). LP was performed in each patient for an average of 12 times. The in-hospital mortality rate from cryptococcal meningitis was significantly reduced from (37/107) 35% to 23% (38/165) after adoption of the guidelines ($p < 0.05$).
Conclusions: Mortality of HIV patients with severe cryptococcal meningitis can be successfully reduced by prompt and aggressive interventions for lowering the highly elevated ICP.

O172 Comparisons of *Aspergillus* galactomannan antigen levels in human immnunodeficiency virus-infected patients with *Penicillium marneffei* infection and cryptococcosis

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Objective: The levels of *Aspergillus* galactomannan (GM) antigen have not been investigated in other endemic fungal infections in human immunodeficiency virus (HIV)-infected patients before. This study aimed to compare the levels of *Aspergillus* GM antigen in HIV-infected patients with *Penicillium marneffei* infection and cryptococcosis.
Methods: Stored sera of 14 HIV-infected patients with *P. marneffei* infection (9 [64.3%] with fungaemia and 7 [50%] with cavitary lung lesions) and 22 HIV-infected patients with cryptococcosis (14 [63.6%] with fungaemia and 8 [36.4%] with cavitary lung lesions) diagnosed between January 2000 and October 2006 were subjected to *Aspergillus* GM antigen testing using a commercial kit. Medical records of those patients with invasive fungal infections were reviewed to exclude the possibility of aspergillosis and concurrent use of antibiotics ever reported to interfere the interpretation of GM antigen test. The median time between diagnosis of invasive fungal infection and collection of serum samples was 0 day (range, −7 to 6 days) for penicilliosis and 1 day (range, 0 to 6 days) for cryptococcosis.
Results: HIV-infected patients with penicilliosis had a statistically significantly higher optic density (O.D.) index of *Aspergillus* GM antigen (median 3.19; range, 0.158–>20) than those with cryptococcosis (median 0.247; range, 0.112–3.849) ($p < 0.0001$). Of the patients with penicilliosis, patients with fungaemia had a statistically significantly higher O.D. index of *Aspergillus* GM antigen (median 10.48; range, 0.401–>20) compared with patients without fungaemia (median 0.378; range, 0.158–4.419) ($p < 0.0001$). Of the patients with cavitary lung lesions but without fungaemia, the median O.D. index of *Aspergillus* GM antigen was also significantly higher in penicilliosis than in cryptococcosis (1.009 [range, 0.247–4.419] vs 0.227 [range, 0.112–3.849]; $p = 0.009$).
Conclusion: Our study suggest that O.D. index of *Aspergillus* GM antigen is elevated in HIV-patients with penicilliosis when compared with those patients with cryptococcosis.

O173 Detection of zygomycete-specific biological markers in BAL fluid of patients with suspected invasive fungal infection

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Objectives: The incidence of invasive zygomycosis (IZ) appears to be increasing, especially in patients treated for haematological malignancy. Presently, no biological markers exsist that facilitate early diagnosis, and one has to rely on conventional diagnostic methods, such as culture, which lack sensitivity. We investigated the presence of zygomycete-antigen (zAg) and zygomycete DNA in bronchoalveolar lavage fluid (BALF) of patients with documented IZ and of those at risk for this disease.
Methods: BALF of 18 neutropaenic patients, who underwent a bronchoscopy on suspicion of invasive fungal infection (IFI), was investigated for the presence of water-soluble somatic zAg by immunoblotting with a commercially available monoclonal antibody (anti-Rhizomucor, Dakocytomation, Denmark). Five of 18 patients had proven IZ. Of the remaining 13 patients without documented IZ, 8 had proven or probable invasive aspergillosis (IA). BALF from 10 non-haematology patients was used as control. BALF samples were also investigated for presence of zygomycete DNA by PCR, using 18S primers.
Results: The BALF of the 5 patients with proven IZ were positive for zAg. In the remaining 13 neutropaenic patients, zAg was detected in BALF of 7. Four of these had probable or proven IA The number of zAg positive BALF from neutropaenic patients with suspected IFI was higher than in the control group (7 of 13 versus 1 of 10, $P < 0.002$). Zygomycete DNA was detected in 11 of 12 BALF samples positive for

zAG, and all BALF samples negative for zAg were also negative for zygomycete DNA by PCR.
Conclusion: zAg and zygomycete DNA are present in BALF of patients with IZ, and might be a useful tool for early diagnosis. The presence of both markers in BALF samples from high-risk patients without a clinically manifest disease might indicate the presence of colonisation or subclinical infection.

Slime wars: an in-depth look at biofilms

O174 Growth, adhesion and biofilm formation of *Pseudomonas aeruginosa* with antibiotic-induced morphological changes under the influence of dynamic conditions

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Objectives: The aim of this study was to investigate the effect of dynamic conditions and antibiotic-induced morphological changes on growth, adhesion and biofilm formation ability of *Pseudomonas aeruginosa*.
Methods: A modified microtitre plate assay was used to quantify adhesion, biofilm formation and planktonic culture density of *Pseudomonas aeruginosa* ATCC 27853 under the effect of 0.5 Minimal Inhibitory Concentration (MIC) of Piperacillin/Tazobactam, Imipenem and Meropenem. Hydrodynamic conditions were obtained by orbital shaking at 250 rpm with the presence of a glass bead in each microtitre well.
Results: These conditions decreased the adhesion and biofilm formation abilities of bacteria with antibiotic-induced morphological changes in comparison to static conditions. The planktonic culture density significantly correlates with adhesion but not with biofilm formation.
Conclusion: Our results demonstrate the importance of using a high-throughput dynamic model to assess the adhesion and biofilm formation deficient behaviour of *P. aeruginosa* with antibiotic-induced morphological changes and suggest the possible use of sub-MIC antibiotics in clinical applications to prevent infections acquired by haematogenous spread. This dynamic model contributes to a better simulation of in vivo conditions of adhesion and biofilm formation of *P. aeruginosa* with altered morphologies induced by β-lactam antibiotics.
Acknowledgments: This study was supported by a grant from Fundação Calouste Gulbenkian (Ref. 47273).

O175 Differences in biofilm development by non-pigmented rapidly growing mycobacteria using different culture media

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Objective: To evaluate the ability of different species of nonpigmented rapidly growing mycobacteria to develop biofilm in different culture media using a microtiter plate assay.
Methods: Collection strains (*Mycobacterium fortuitum* ATCC 6841 and ATCC 13756, *M. chelonae* ATCC 19235 and ATCC 35752, *M. abscessus* DSM 44196, *M. peregrinum* ATCC 14467, *M. mucogenicum* DSM 44124, *M. septicum* ATCC 700731, *M. immunogenum* ATCC 700505, *M. mageritense* ATCC 700351, *M. porcinum* ATCC 33776, *M. elephantis* DSM 44368, *M. smegmatis* CECT 3032, CECT 3020 and CECT 3017, *M. goodii* ATCC 700504, *M. alvei* CECT 3021, and *M. brumae* CECT 3022) of non-pigmented rapidly growing mycobacteria (NPRGM) were inoculated in tissue culture-treated polystyrene microtiter plates and incubated in Middlebrook 7H9, PBS-5% glucose or sterile tap water. Plates were incubated at room temperature with rotation during 70 days, and media were changed each 4 days. One well for each strain and medium was stained with Fuchsin, decoloured with ethanol, and photographed to measure the biofilm development the days 1, 4, 8, 12, 20, 27, 34, 41, 48, 55, 62 and 70. Calculation of the surface covered by bacteria was performed analysing the photograps with the ImageJ software for Windows.
Results: All the strains tested were able to form biofilm in all the media tested. However, differences were detected between the different media. The isolates were able to form biofilm faster in Middlebrook 7H9 (100% of surface covered in day 28 for all the strains) than in tap water (100% of surface covered in day 63 for all the strains). The day 70 none of strains covered all the surface when using PBS-5% glucose. No difference was found between species in biofilm development.
Conclusions: All the NPRGM tested were able to develop biofilm in all tested media. The biofilm development is dependent on the type of the culture media, being faster when a rich media is used. No differences were detected in biofilm development among the different species.
Acknowledgments: T.J. Kinnari was funded by a grant from the Academy of Finland. N. Zamora was funded by a grant from the Fundación Conchita Rábago de Jiménez Díaz (Madrid, Spain).

O176 Effect of Fe and glucose on the glycolysis and gluconeogenesis in biofilm-associated *Staphylococcus epidermidis*

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Objective: To determine the role of GapA (glycolysis) and GapB (gluconeogenesis) in *Staphylococcus epidermidis* biofilms.
Methods: A biofilm-forming *S. epidermidis* strain from a proven catheter-related infection was used. For in vitro studies, to examine the influence of glucose, bacteria were grown overnight in BHI and re-incubated in BHI (glucose-rich; Fe-rich) or 0.9% NaCl (glucose-limited; Fe-rich). To examine the influence of iron (Fe) bacteria were grown overnight in RPMI without Fe (fRPMI) and re-incubated in fRPMI (glucose-rich; Fe-limited) or fRPMI with 25 μM Fe (glucose-rich; Fe-rich). For in vivo studies a subcutaneous rat model was used. Quantification of bacteria and gene expression through Taqman PCR were performed as described by S. Vandecasteele et al. (Biochem. Biophys. Res. Commun. 2002; 291: 528–534). Extracellular matrix and bacteria were visualised through confocal laser scanning microscopy (CLSM).
Results: In vitro, in a glucose-rich environment the expression of gapA and gapB did not differ between planktonic and sessile bacteria and stayed constant over time. Both in planktonic and sessile bacteria, expression of gapA was up-regulated in comparison to gapB. In vitro, in a glucose-limited environment, expression of gapA in sessile bacteria decreased in comparison to its expression in planktonic bacteria and expression of gapB in sessile bacteria increased to the expression level of gapA in sessile bacteria.
In vitro, in sessile bacteria, gapB expression was high in a medium with a low Fe content, irrespective of the glucose content. Simultaneously with the increased expression of gapB, PIA production could be visualised through CLSM in sessile bacteria in all media.
In vivo expression of gapA was high and remained constant over a period of two weeks. The expression of gapB decreased during the initial phase of implantation, but reached the expression level of gapA after two weeks of implantation.
Conclusion: The persistent expression of gapA in sessile bacteria could indicate a role in biofilm formation, especially in the early stages, while expression levels of gapB could indicate a role in the later phases of biofilm formation. Results of gene expression and CLSM indicate a link between gapB and PIA production that is influenced by both Fe and glucose.

O177 Biofilm formation may be an independent virulence factor in wild-type *Staphylococcus saprophyticus* strain 7108 in contrast to wild-type strain CCM 883

F. Szabados, K. Strate, M. Kaase, T. Sakinc, A. Anders, S. Gatermann (Bochum, DE)

S. saprophyticus is second only to *E. coli* the most important causative organism of uncomplicated urinary tract infection in young female outpatients. Compared to *S. aureus* and *S. epidermidis* only very few virulence factors in *S. saprophyticus* have been described, for

example Ssp, a surface-associated lipase, Aas, an autolysin adhesin, SdrI, a collagen binding protein, and urease activity. Purportedly, the uropathogenicity of *S. saprophyticus* can be attributed to its ability to cope with the high range of variation in salt- and urea-concentration in human urine. The aim of this study was to elucidate virulence factors of uropathogenicity.
Methods: Bacterial growth was examined under different conditions using a modified model of artificial urine as previously described. Bacterial aggregation was observed in bright light and electron micrographs. Biofilm formation was tested using native polystyrene and crystalline pre-coated microtiter plates.
Results: In *S. saprophyticus* strain CCM 883, in contrast to wild-type strain 7108, generation-time was increased. In *S. saprophyticus* wild-type strains 7108 and 9325, bacterial aggregation appeared near large crystal structures in contrast to its urease negative derivative strain GJ1187 and the wild type strain CCM 883. In native polystyrene and crystalline pre-coated microtiter plates, biofilm formation was observed in strain 7108 in contrast to strain CCM 883. The biofilm formation in *S. saprophyticus* seems independent of agr-, Ssp-, SdrI, since no difference between knockout mutants to wild type strain was observed.
Conclusion: Bacterial aggregation could be due to increased adhesion. The lipase activity may modulate hydrophobicity and Ca^{2+} binding. Higher local Ca^{2+} concentration, as well as strengthened bacterial aggregation, may lead to increased crystal formation. Bacteria may adhere to crystals, additionally to the later mechanism due to biofilm formation. In crystallisation process, bacteria were embedded into the crystal structure. This may be a new model of infectious stone genesis. This study strongly suggest that *S. saprophyticus* wild-type strain CCM 883 lacks important virulence factors in contrast to wild-type strain 7108 and biofilm formation may play an important role in *S. saprophyticus* urinary tract infection.

O178 Production of an anti-biofilm molecule by *Candida albicans* yeasts growing as a biofilm

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Objectives: The influence of biofilm formation in catheter-related candidiasis has been established and it has been shown that the development of biofilm by the colonising yeasts confers resistance to antifungals. The purpose of this work was to demonstrate the production, by the *Candida albicans* yeasts growing as a biofilm, of a molecule able to reduce biofilm growth.
Methods: An in vitro model of *C. albicans* biofilm associated with 100% silicone catheters was used. The supernatant medium recovered from *C. albicans* (ATCC 3153) biofilm aged of 24 hours was added during the course of a new biofilm formation made with 4 *C. albicans* strains (182, 444, 2091 and ATCC 3153), and its influence was subsequently evaluated with XTT method. Ultrafiltration and purification assays to characterise the modulating fungal molecule responsible for the biofilm supernatant activity are presented in this study.
Results: The addition of the supernatant to adherent *C. albicans* cells induced a significant ($p < 0.001$) inhibition of the biofilm growth. This activity was not observed when the supernatant was added to preformed biofilms. Ultrafiltration and purification assays demonstrated that this molecule is small (<3000 Da) and hydrophilic.
Conclusions: These preliminary results suggest that this new molecule, naturally produced within yeasts communities, could be a good candidate for the prevention of biofilms associated with indwelling medical devices.

New diagnostic kits on the block

S179 Finding the niche of T cell assays in tuberculosis infection and disease

P. Hill (Banjul, GM)

In 1994 the first article describing an 'early secreted' antigen (esat-6) from the culture filtrate of *M. tuberculosis* was published. Three key properties of this antigen are that it is highly immunogenic, it is not found in BCG or many environmental mycobacteria, and it is a key virulence factor. Its immunogenicity and specificity led to a series of studies to assess its potential as a major component of new diagnostic tests. Such tests have evolved into two major forms-a whole blood assay marketed under the name Quantiferon and an ELISPOT assay marketed under the name T-spot. New antigens have been assessed for improved test performance, most notably CFP-10. Studies of in-house versions of these assays, and more recently the commercial kits, have shown encouraging results in TB cases for the diagnosis of disease and in their close contacts for the diagnosis of *M. tuberculosis* infection. It is clear that these tests are not confounded by prior BCG vaccination and are probably not subject to boosting by injected tuberculin. Their quantitative readouts may also provide important information with respect to infectious load. However, some findings have caused concern: disparity in test performance between temperate non-TB endemic and tropical TB-endemic settings, evidence of relatively poor sensitivity to the presence of certain *M. tuberculosis* strains (eg. *M. africanum*) and in certain sub-groups (eg. Pulmonary TB, certain TB contacts), and rapid unexplained test reversion. It is therefore becoming clear that T cell assays may have a niche in the diagnosis of certain TB cases and in certain individuals suspected of having *M. tuberculosis* infection, but this niche has not yet been fully defined. More longitudinal studies, especially following TB case contacts for the development of disease, are indicated. Also more studies should be undertaken in TB endemic areas. Finally, if these tests are to have any role in TB control globally they would need to be much cheaper and more easy to use. Some advances with respect to these two issues have already taken place.

Antiretroviral treatment

S184 New targets for innovative HIV treatment

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Over the last ten years unprecedented progress has been made in the treatment of HIV infection by the application of combination antiretroviral therapy (cART). Three classes of drugs have been contributed to this success: the nucleoside (NRTI) and the non-nucleoside (NNRTI) reverse transcriptase inhibitors, and the protease inhibitors (PI). With the advent of the fusion inhibitor enfuvirtide (T20) the field has been opened for new classes of drugs. During the last year a whole bundle of drugs with new targets has been studied in humans. Among the most developed compounds are CCR5 antagonists and integrase inhibitors. Other classes in earlier phases of clinical development include attachment inhibitors and maturation inhibitors.
It is expected, that integrase inhibitors and CCR5- antagonists will be introduced into clinical practice in 2007/2008. Both of these drug classes have been first studied in heavily treatment experienced patients having failed NRTI, NNRTI and PI therapy. In preliminary analyses the integrase inhibitor MK-0518 combined with optimised background treatment (OBT) has shown clinical superiority over OBT alone. Up to 72% of patients treated with MK-0518 have reached complete viral suppression (HIV-RNA <50 c/mL) after 16 weeks. Preliminary data also suggest a high virological potency of the drug in treatment naïve patients. MK-0518 was applied bid and was well tolerated in both pretreated an treatment naïve patients.
Two CCR5 antagonists are currently studied in clinical trials, maraviroc and vicriviroc. Vicriviroc together with OBT has shown superiority over OBT alone in treatment experienced patients, but has failed in a phase II study in treatment naïve patients presumably due to matters of dosing. Further studies to define the optimal dose (together with ritonavir boosting) are underway. With maraviroc results of a study in treatment experienced patients exhibiting dual tropic virus (R5/X4) are available. In this population, maraviroc did not show additional virological efficacy over placebo, but lead to a greater increase of CD4 cells.
In conclusion, new drugs in new classes improve the treatment options for patients with multi-resistant HIV considerably. Since these drugs

seem to be very well tolerated there is also a potential for their use in treatment naïve patients in the future.

S185 Challenges in the management of adverse effects of long-term ART

A.M. Geretti (London, UK)

Antiretroviral therapy is highly effective in controlling HIV replication, inducing immune reconstitution and preventing AIDS-related morbidity and mortality. Current antiretroviral regimens however cannot achieve virus eradication and virological rebound virtually always occurs after therapy discontinuation. Long-term highly active antiretroviral therapy (HAART) is life saving, but poses several challenges. Potential adverse events can be categorised as problems related to adherence, pharmacokinetics, toxicity and drug resistance. Various interventions have been explored to improve and maintain good adherence among treated individuals, ranging from treatment simplification strategies to addressing mental health problems, and a major challenge is related to the need for integrated biomedical, social and behavioural interventions. Inadequate adherence however accounts for only a subset of sub-therapeutic drug levels in clinical practice. There is a significant degree of inter-individual variability in absorption, metabolism and cellular transport of antiretroviral drugs, with potential impact on both efficacy and toxicity of therapy. Important determinants include gender, age, ethnic origin, and genetic polymorphisms. Although evidence of the influence of pharmacogenomics is growing, there remain limited data to guide clinical practice. Proton pump inhibitors are an example of commonly prescribed medications that can affect antiretroviral drug levels by reducing the absorption of certain protease inhibitors (PIs) and increasing exposure to others. Herbal medicines such as St. John's wort, widely used by HIV patients, also have the potential to interact with antiretroviral drugs. Thus, patient education and good communication across care providers are paramount to prevent unfavourable drug–drug interactions.

The risk of toxicity associated with long-term antiretroviral therapy is difficult to predict reliably in an aging population of HIV-infected persons. Major examples of HAART-related toxicity include mitochondrial damage and lipoatrophy associated with the use of nucleos(t)ide reverse transcriptase inhibitors (NRTIs), and fat accumulation, dyslipidaemia and insulin resistance associated with the protease inhibitors (PIs). The risk of cardiovascular disease is a particular concern, due to the combined effects on the cardiovascular system of HIV infection, HAART and life-style related risk factors such as smoking which are common among HIV-infected persons. In addition, renal, hepatic and bone toxicity represent emerging side effects of long-term treatment.

Patients starting therapy on recommended first-line regimens achieve a high degree of virological and immunological success. However, drug resistance continues to emerge as a result of problems with adherence and tolerability. Furthermore, there remain a large number of HIV-infected persons who have accumulated drug resistance through mono and dual therapy in the pre-HAART era and the suboptimal use of therapy in the early HAART era. In addition, a substantial proportion of newly infected persons acquire drug-resistant virus.

New drugs and drug classes are required to optimise treatment among drug-experienced persons and reduce the risk of side effects. Several new compounds have recently entered clinical use that show improved tolerability profile or activity against drug resistant virus. Improved methods for detecting and interpreting drug resistance are also being developed to adequately guide treatment. In spite of these concerns however, the benefits of HAART far outweigh the risk of adverse events.

Clinical significance of innate immune defence

S188 *Leishmania major* induces secretion of inflammatory cytokines and chemokines by keratinocytes via a TLR2 pathway

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Objectives: Some features of the cutaneous pathologies observed in patients with cutaneous leishmaniasis can be reproduced in the murine model of infection with *L. major*. After subcutaneous injection of parasites in C57BL/6, mice are resistant to infection; in contrast BALB/c mice are susceptible to infection. Resistance and susceptibility to the infection were related to the development of polarised Th1 and Th2 response. Keratinocytes are the first effector cells encountering parasites during infection with *L. major* but their role in the initiation of the immune response is mainly ignored. The aim of this study is to analyse the role of the interaction between *L. major* and keratinocytes in the subsequent immune response induced by infection with this parasite.
Methods: Cultured primary murine keratinocytes from neonatal C57BL/6 and BALB/c mice (1–3 days old) were stimulated in vitro by *L. major* LV39 strain (MHRO/Sv/59/P strain), and mRNA expression and secretion of inflammatory cytokines (IL-1, IL-6 and TNF-a) and chemokines (MIP-2) were analysed.
Results: Parasites adhere to keratinocytes through the flagellar tip, the flagellar base or with the posterior pole in both strains of mice, but they never infect keratinocytes. Whereas this interaction induces an up-regulation of IL-1 and MIP-2 mRNA expression and secretion in both BALB/c and C57BL/6 mice, IL-6 is produced in response to *L. major* only in BALB/c mice. Furthermore, we demonstrated that TLR-2 –/– mice are unable to produce MIP-2 in response to *L. major* stimulation.
Conclusion: The results strongly suggest that keratinocytes are able to produce cytokines in response to *L. major* stimulation through in part-TLR-2.

S189 Innate immunity and *Aspergillus* infections

L. Romani (Perugia, IT)

Aspergillus fumigatus, a termotolerant saprophyte, is associated with a wide spectrum of diseases in humans, that includes saprophytic colonisation of preexisting cavities, allergic asthma, allergic bronchopulmonary aspergillosis occurring as a complication of bronchial asthma or cystic fibrosis, and invasive aspergillosis in immunocompromised patients. Immunocompetent and non-atopic subjects are relatively resistant to *A. fumigatus* diseases and disease occurs in the setting of host damage. The inherent resistance to diseases caused by *A. fumigatus* suggests the occurrence of regulatory mechanisms that provide the host with adequate defence without necessarily eliminating the fungus or causing unacceptable levels of host damage. Respiratory tolerance is mediated by plasmacytoid dendritic cells producing IL-10 and inducing the development of CD4+ T regulatory cells expressing FoxP3 and regulating inflammation and allergy through the indoleamine 2,3-dioxygenase (IDO)-dependent pathway. Thus, the IDO-dependent pathway has a unique e central role in this process as it participates in the effector and inductive phases of immunity and tolerance to the fungus.

S190 Intracellular inhibitors of innate immunity encoded by Vaccinia virus

G.L. Smith (London, UK)

Vaccinia virus (VACV) is the vaccine that was used to eradicate smallpox. Like other poxviruses, VACV is a large DNA virus that replicates in the cytoplasm, produces multiple infectious forms of virus and encodes many virulence factors that are non-essential for virus replication in cell culture. Many of these virulence factors are

encoded in the terminal regions of the virus genome and are called immunomodulators because they interfere with the host response to infection. Some of these immunomodulators are present only within infected cells and inhibit signalling pathways induced by ligand engagement of cytokine, interferon or toll like receptors. Other molecules inhibit pathways leading to the induction of apoptosis. This talk will describe proteins from VACV that inhibit apoptosis or intracellular signalling pathways leading to the induction of interferon or pro-inflammatory cytokines. These studies will illustrate how studying the structure and function of these molecules is increasing our understanding of virus pathogenesis and cell biology.

Current trends in parasitology

S191 European consensus on malaria chemoprophylaxis

C. Hatz (Basle, CH)

Every year several thousand malaria cases with a mortality around 1% are reported in Europe. Visiting friends and Relatives (VFRs) are at particular risk.

The complex situation of malaria transmission in various endemic areas requires differentiated recommendations for malaria prophylaxis. Malaria prophylaxis comprises of multiple components. Malaria risk can be reduced by compliant exposure prevention, and with chemoprophylactic drugs for travel to high risk areas are available.

Key points for malaria prevention and management include awareness in endemic regions and after return, avoid mosquito bites, compliance with chemoprophylaxis, and seeking immediate diagnosis and therapy in case of fever.

The importance of exposure prophylaxis should be emphasized.

It is recommended to use mosquito repellents after dusk, especially if outdoor activities are performed. Light-coloured, loose-fitting insecticide-treated clothing with long trousers and long sleeves are suggested. Sleeping under insecticide treated bed nets or in air-conditioned rooms which are pre-treated with insecticides (knockdown spray) is recommended.

Chemoprophylaxis is recommended in high risk areas. In most settings, either mefloquine (Lariam®), atovaquone/proguanil (Malarone®) or doxycycline (monohydrate) are used. In German speaking countries, emergency treatment is recommended for trips to regions with low or intermediate malaria risk. This strategy is recommended when the infection risk is lower than the risk of severe drug side effects.

Good information by the consulting doctor and personal responsibility of the traveller are essential for the correct handling of emergency-self-treatment. Drugs used include artemether/lumefantrine (Riamet®), atovaquone/proguanil (Malarone®) or mefloquine (Lariam®). The guidelines for the application of the emergency-self-treatment should be discussed thoroughly with the traveller, to make sure that in case of fever the correct action will be taken:

1. In case of fever (sudden onset or rapidly progressive) – axillary temperature >37.5ºC (oral, tympanal or rectal >38ºC) – a doctor should be seen and a malaria blood test should be performed. A working thermometer is essential in the tropics.
2. If no doctor can be seen within 24h and the traveller is in an endemic region for at least 6 days, the fever should be lowered.
3. The malaria emergency medication should be taken with adequate amounts of fluid.
4. In every case, also after the intake of the malaria drug a doctor must be consulted at the earliest possible time.

S193 Current trends in echinococcal diseases

P. Kern (Ulm, DE)

The term echinococcosis describes two diseases which markedly differ in their presentation, behaviour and management: Alveolar echinococcosis (AE) is caused by the metacestode *Echinococcus multilocularis* and cystic echinococcosis (CE) by *E. granulosus*. The larval growth of the two bugs is very different and separates the "malignant" AE from the "benign" CE. Thus, current trends are discussed separately.

AE: A recent European initiative (EchinoRisk) discovered a continuous increase and spread of the parasite in the final host (fox). In parallel, human cases were observed in regions outside the known endemic areas, such as in the Baltic States, in Hungary and Slowakia. Risk factors for humans are farming and a long-standing, close contact to dogs. The initially asymptomatic liver disease is still rare in Central Europe, and diagnosis is often made at a late stage of the disease. An anatomical classification system has been used (PNM adapted from the TNM system of tumours), allowing now a comparison of the disorder at the time of first diagnosis in different clinical settings. New foci have been discovered in China/Tibet with a prevalence rate of up to 4% of the affected population. Imaging techniques, such as Ultrasound, CT, MRI and PET/CT contributed to a much better description of the lesions. However, the expertise of the radiologist is often not available due to the low incidence of AE. Serology is helpful, but may be misleading due to non-standardised tests. Molecular tools have been developed, and help histopathologists to clearly separate the two bugs by microscopy. Radical surgery is curative and the first choice of treatment. Due to the potential "malignant" features of the disease palliative surgery and liver transplantation should be strictly avoided. Instead continuous treatment with benzimidazoles is the backbone of a lifelong management of AE.

CE: The disease, also known as hydatid disease, occurs worldwide. Recent trends indicate a decreasing incidence throughout the Mediterranean countries, whereas important new foci have been disclosed in China/Tibet, and re-emergence is obvious in countries of the former Soviet Union. For Central Europe it is believed that CE is an imported disease. However, autochthonous infections are being observed, and underline the fact that transmission is still possible. Based on molecular markers the species of *E. granulosus* was recently further subdivided according to its main intermediate host in *E. granulosus*, *E. ortleppi*, *E. equinus*, *E. intermedius*. In contrast to AE, the larva forms a single or several fluid-filled cyst(s), and induces a well-organised, compact capsule of host origin. Serological diagnosis of the disease in humans is still unsatisfactory, since young and unruptured cysts remain serologically negative. The recently established WHO Classification of hepatic cysts has much improved the clinical management. Experiences with the PAIR/PEVAC procedure are encouraging, and have shown to be safe for hepatic hydatids. Surgery is not anymore the treatment of choice. Instead many centres apply short term cycles of benzimidazoles which act parasitocidal and accelerate the degeneration of the cyst. Others favour the Watch and Wait concept, and carefully observe the natural degeneration of the cyst(s).

S194 Current trends in filarial diseases

A. Hoerauf (Bonn, DE)

Filarial worm infections of humans cause morbidity and even death in developing countries of the tropics. Current antifilarial drug therapies target only the first stage larvae, requiring many years of annual/biannual treatment. Another problem with controlling filarial infections is the lack of any alternative drugs that can be used in the current mass drug administration programmes should resistance develop. Wolbachia, endosymbiotic bacteria that are found in most of the human filarial worms are excellent targets for the discovery of new antifilarial drugs because of their requirement for worm embryogenesis, development and adult survival. In both lymphatic filariasis and onchocerciasis, administration of 6 weeks of doxycline at a dose of 200 mg/day results in a high macrofilaricidal effect of over 80% and 70%, respectively. Targeting of Wolbachia with antirickettsial drugs has lead to the recommendation of doxycycline for use on an individual basis and may be recommended in areas where resistance to current drugs may develop. Evidence that eliminating the endobacteria reduces adverse reactions to current drug therapies and even reduces early stages of lymphatic pathology is also accruing. Research is underway to discover new drugs, preferably those already approved for use in humans, that have anti-wolbachial activity and work in a shorter time and are widely applicable.

Experimental models of infectious diseases

O195 Development of glycopeptide-intermediate resistance by *Staphylococcus aureus* leads to attenuated infectivity in a rat model of endocarditis

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Objectives: Whether *S. aureus* developing intermediate resistance to glycopeptides, i.e. the GISA phenotype, are altered in their infectivity is poorly documented. Here we used an isogenic pair of glycopeptide-susceptible and -intermediate *S. aureus* (M1V2 and GISA M1V16; vancomycin MIC: 2 and 16 mg/l, respectively) to study their ability to (i) adhere to fibrinogen (Fg) and fibronectin (Fn) in vitro, (ii) persist in the bloodstream after intravenous inoculation, (iii) colonise aortic vegetations in rats, (iv) multiply in situ thereafter, and (v) compete for valve colonisation when inoculated in mixed cultures.
Methods: GISA M1V16 was selected by vancomycin exposure of the glycopeptide-susceptible, methicillin-resistant *S. aureus* M1V2 (agr type II) in the laboratory. The phenotypic expression of resistance to vancomycin and the stability of the phenotype was analysed by population analysis. Adherence was tested on immobilised Fg and Fn. Rats with catheter-induced aortic vegetations were inoculated with 10^3–10^6 CFU of the test strains. Rats were killed after 24 h. Vegetations and spleens were cultured and the lower inoculum infecting 90% of vegetations (ID90) was recorded. Blood cultures were drawn within the first 60 min. Infectivity was also compared by co-inoculation with 10× the ID90 of M1V16 and a rifampin-resistant variant of the parent M1V2, to discriminate them in organ cultures.
Results: The GISA M1V16 mutant grew on plates containing 16 mg/l of vancomycin and its resistance phenotype remained stable after 5 passages on drug-free medium. Both GISA and parent strains adhered similarly to Fg and Fn in vitro. In rats, the GISA M1V16 was cleared faster from the blood ($P < 0.005$) and required 100× more bacteria than the parent (10^6 versus 10^4 CFU) to infect 90% of animals. GISA M1V16 had also 100–1000× lower bacterial densities in vegetations and spleen. After co-inoculation, M1V2 and GISA M1V16 produce similar vegetation densities early after injection. However, while the vegetation counts of the parent increased at 48, 72 and 120 h, the counts of GISA M1V16 remained stable.
Conclusions: GISA showed attenuated pathogenicity (ID90) and fitness (intra-vegetation growth) in rats with experimental endocarditis. Thus, the GISA phenotype is globally detrimental to infectivity. This might explain its clustering in immuno-compromised patients. Whether a very prolonged exposure to glycopeptides might restore pathogenicity and fitness defects remains to be determined.

O196 Evaluation of moxifloxacin and levofloxacin against *S. pyogenes* in various animal models

R. Endermann, K. Ehlert (Wuppertal, DE)

Objectives: Group A streptococci are frequently implicated in complicated skin and skin structure infection (cSSSI). Moxifloxacin (MXF) has recently been approved for this indication. In this study, the therapeutic efficacy of MXF or levofloxacin (LFX) against a clinical isolate of *S. pyogenes* (6A47 wild-type) was investigated. We studied both quinolones in a murine sepsis model and in two cSSSI models: the granuloma pouch and the Gelfoam® implant model.
Methods: *Sepsis model*: bacteria were suspended in 5% mucin in saline and inoculated intraperitoneally. Antibiotics were given intravenously (i.v.) at 1, 4, 24 and 32 h post-infection (p.i.; 5 animals/group). Survival was monitored for 5 days. Granuloma pouch model: pouches were formed by injecting 5 mL of air and 0.5 mL of 0.1% croton oil in olive oil under the skin of the back. After 72 h, the air was replaced by 1 mL of 0.25% agar in saline. A bacterial suspension (0.5 mL) was injected into the pouch after 48 h. Antibiotics were given i.v. at 0.5, 4, 24 and 32 h p.i. (7 animals/group), and viable counts in exudates were assessed at 48 h.
Gelfoam® model: a collagen piece was implanted under the skin of the back. After 3 days, 50 µL of bacterial suspension was injected into the implant. Antibiotics were given i.v. for 3 days (b.i.d.), starting 2 h p.i. (5 animals/group); implants were removed for bacterial cell counts 24 h after treatment ended.
Results: Sepsis model: 100% survival was achieved with 25 mg/kg MXF, compared to 40% survival with 25 mg/kg LFX. Granuloma pouch and Gelfoam® models: the colony forming unit (CFU) reductions achieved with MXF were greater than those achieved with LFX (Table).

CFU reduction (log_{10}) in the granuloma pouch and Gelfoam® models

	MXF	LFX	P^a
Granuloma pouch (log CFU reduction[b])			
50 mg/kg	–0.49	–0.18	0.48
75 mg/kg	–2.31	–0.30	0.009
Gelfoam® (log CFU reduction[b])			
25 mg/kg	–1.56	–0.92	0.19
50 mg/kg	–3.11	–1.38	0.03

[a]Mann–Whitney test; [b]Log CFU reduction in relation to the untreated control group.

Conclusion: In the murine sepsis model as well as in two models of cSSSIs, MFX showed good antimicrobial activity against a recent clinical isolate of *S. pyogenes*.
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O197 Virulence and biofilm formation of non-mucoid and mucoid *Pseudomonas aeruginosa* strains in cystic fibrosis: an animal study

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Most adult cystic fibrosis (CF) patients are chronically infected with *P. aeruginosa* in their lungs. Two major features of *P. aeruginosa* are the abillities to form biofilms and become mucoid. Recently, we reported a time-dependent reduction in the ability to form biofilm by non-mucoid *P. aeruginosa* isolates from the same CF patient obtained over a period of 23 years. In order to investigate whether the declined ability of the PFGE-identical isolates to form biofilm in vitro correlated to pathogenicity in vivo, 5 non-mucoid clones from 1980 and 1988 (early clones), and 1997, 1999 and 2003 (late clones) embedded in alginate were installed in the lungs of five groups of BALB/c mice. Mice were sacrificed after 5 days of infection. The experiment was evaluated by macroscopic lung pathology, histopathology, quantitative bacteriology (CFU/lung) and pulmonary cytokine production.
Survival was significantly higher in mice infected with the late clones, as compared to mice infected with the early clones ($p < 0.025$). Macroscopic pathology was more disseminated in mice infected with the early clones as compared to mice infected with the late clones ($p < 0.0015$). Inflammation involving polymorphonuclear neutrophils (PMN's) was present more often in mice infected with the early clones as compared to mice infected with the late clones ($p < 0.05$). In addition, a higher degree of inflammation as well as more mice with athelectasis was observed after infection with the early clones ($p < 0.05$). CFUs were higher in mice infected with the early clones as compared to mice infected with the later clones ($p < 0.04$). Pulmonary TNF-α, IL-10 and GM-CSF increased in mice infected with the late clones, whereas the mobiliser of PMNs from the bonemarrow G-CSF and the PMN chemoattractant MIP-2 decreased in mice infected with the late clones.
Mucoid PFGE-identical isolates from 1988, 1997 and 2003 from the same patient were installed in the lungs of BALB/c mice. Survival decreased significantly in mice infected with the late clones from 1997

(all 23 mice died) or 2003 (21 of 23 mice died) as compared to the clone from 1988 (1 of 24 mice died) ($p < 0.005$).
In conclusion, non-mucoid *P. aeruginosa* lung infection is characterised by a time-dependent correlation between the ability to form biofilm, the ability to establish lung infection, and the inflammatory responses. In contrast, pathogenecity of the mucoid strains increases with time supporting the importance of controlling this phenotype by all means.

O198 Reduction of pathogen shedding and alleviation of disease signs in pigs challenged with *Salmonella* Typhimurium by the application of a five-strain probiotic combination

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Objectives: Infection with *Salmonella* spp. is a major cause of gastroenteritis, with many thousands of cases reported every year. The use of probiotics offers the potential to improve this situation. Here, we investigate the effect of oral treatment of pigs with a defined lactic acid bacteria culture mixture on both clinical and microbiological signs of *Salmonella* Typhimurium infection.
Methods: Fifteen weaned pigs blocked by gender and weight were administered skimmed milk (as a control) or a mixture of five probiotic strains (given as either a milk fermentate or milk suspension) for a total of 30 days. The mixture comprised two strains of *Lactobacillus murinus* and one strain each of *Lb. salivarius* ssp. *salivarius*, *Lb. pentosus* and *Pediococcus pentosaceus*. Following probiotic administration for six days, animals were orally challenged with *Salmonella* Typhimurium; the health of the animals and the microbiological composition of their faeces were monitored for 23 days post infection.
Results: Animals treated with probiotic displayed reduced incidence, severity and duration of diarrhoea. These animals also gained weight at a greater rate than control animals and mean faecal numbers of *Salmonella* were significantly reduced in probiotic-treated animals at 15 days post infection.
Conclusions: The probiotic mixtures used lead to an amelioration of clinical signs in *Salmonella* Typhimurium-infected pigs early in the course of infection, and significantly reduced pathogen shedding over a longer timeframe. While the exact mechanism remains to be fully elucidated, this demonstrates the validity of using commensal lactic acid bacteria strains in the prevention of gastrointestinal infection. The in vitro and in vivo procedures used to isolate and select the bacteria are also validated. While the probiotics examined in this study are of obvious interest to those involved in the pig production industry, the similarities between the pig and human gastrointestinal tracts suggest that the probiotics offer potential in cases of human salmonellosis, given that the methodology used is also likely to be applicable to human disease control.

O199 Efficacy of linezolid in the treatment of biofilm-associated infection using a rabbit model of methicillin-resistant *Staphylococcus aureus* catheter biofilm

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Objectives: Catheter-related biofilm infections (CRBI) are a major cause of bloodstream infections in the USA. One of the most common causes of CRBI related bloodstream infection is methicillin-resistant *Staphylococcus aureus* (MRSA). Catheter removal has become an integral part of the therapeutic approach to CRBI, however, it is associated with increased medical costs, potential morbidity, and is not always feasible in critically ill patients. Antibiotic lock therapy provides the means for local delivery of high drug concentrations to overcome resistance due to bacterial biofilms that coat the catheter surface. In this in vivo study, we compared the effectiveness of linezolid (LIN) versus vancomycin (VAN) antibiotic lock therapy against MRSA CRBI.
Methods: In New Zealand White rabbits, silicone catheters were tunneled subcutaneously and surgically placed in their jugular vein. The catheter lumen was infected by locking 1×10^7 MRSA in the lumen for 24 h. To maintain catheter patency, heparin flushes were performed daily. Rabbits were randomised into three groups: untreated control, VAN 2 mg, and LIN 2 mg. Each drug was locked in the catheter lumen for 8 h a day for 7 days. After treatment, the animals were anaesthetised and peripheral blood cultures were taken from the catheter. Animals were sacrificed, catheter removed and divided into two pieces; one piece for quantitative culture and the other for scanning electron microscopy (SEM).
Results: Biofilms were confirmed by blood culture showing MRSA growth from the catheter 3 days post-infection. Catheters removed from untreated controls yielded a higher fungal burden than LIN and VAN-treated groups (2.59 ± 0.76, 0.20 ± 0.08, and 0.18 ± 0.68, respectively). No significant difference was observed between LIN and VAN treatment groups, (P value 0.8764). SEM of untreated control catheters revealed patchy, abundant biofilms while both LIN and VAN treated catheters had almost completely cleared surfaces. VAN-treated catheters had small patches of bacteria with damaged biofilm matrix, while LIN-treated catheters had scattered patches of bacteria with little or no surrounding matrix.
Conclusion: Antibiotic lock therapy with LIN may be a useful treatment strategy against MRSA in CRBI's. A prospective, randomised clinical trial evaluating LIN for the treatment of MRSA caused CRBI is warranted.

Molecular epidemiology of resistance genes and strains

O200 Identification of plasmid-mediated quinolone resistance in *Salmonella enterica* isolated in England and Wales

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Objectives: Plasmid-mediated quinolone resistance (qnr) genes have been identified in enterobacteria from several countries. Such resistances have frequently been associated with strains that produce extended-spectrum β-lactamases (ESBLs). As *Salmonella* with resistance to quinolone antimicrobials have become increasingly common in isolates from humans in England and Wales, a study has been initiated to investigate the occurrence of such genes in isolates from humans and food, and to assess their importance for public health.
Methods: A panel of 118 isolates of *S. enterica* resistant to nalidixic acid and with concomitant decreased susceptibility to ciprofloxacin (MIC: 0.25–1.0 mg/L), from cases of human infection and from foods in England and Wales, were screened for the presence of qnrA, qnrB and qnrS by PCR. Isolated qnr plasmid was tested by PCR genes and for ESBL genes, and for 18 commonly occurring replicons. All strains positive for qnr genes were further characterised by PFGE.
Results: All isolates were negative for qnrA and qnrB; 6 isolates belonging to four serovars (S. Stanley, 2; S. Typhimurium, 1; S. Virginia, 1; S. Virchow, 2) were positive for qnrS. Of these the isolates of Stanley and Typhimurium were from patients infected abroad, the isolate of Virginia was from a patient who had not travelled abroad, and the two isolates of Virchow, one from imported frozen chicken and one from a patient were associated with a series of infections in 2003/2004. qnr plasmids were transferable either by conjugation or transformation; their molecular masses ranged from 13.5 kb (S. Virginia) to >148 kb (S. Stanley). Four of the six plasmids coded for additional resistance, in two cases for resistance to 10 additional antimicrobials. The qnr plasmids from the S. Virchow isolates were of similar size and conferred a similar resistance phenotype as the first qnr plasmid, identified in *Shigella flexneri* in Japan.
Conclusions: These results indicate that plasmidic quinolone resistance, often linked to other resistances, has now appeared in *S. enterica* in Britain. This is of particular concern for the treatment of infections with invasive serovars such as S. Virchow.

O201 Development of a low-cost and robust assay for the screening of multiple genetic loci in *Mycobacterium tuberculosis*

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Objective: The rapid characterisation of (drug-resistant) *Mycobacterium tuberculosis* (MTB) would be useful for the research and treatment of tuberculosis (TB). Many important genetic markers of MTB have been identified, notably for drug resistance, but also genotype, bacterial lineage and adaptive potential. A single assay allowing identification of these markers would aid control efforts. Multiplex PCR is unsuitable for simultaneous amplification of several genetic loci in one reaction. With Multiplex Ligation-dependent Probe Amplification (MLPA) only one primer-pair is used for the amplification of multiple genetic targets. We explored the utility of MLPA as a screening tool to rapidly characterise MTB-strains

Method: MLPA can identify multiple single nucleotide polymorphisms (SNPs) by amplification of sequence-specific MLPA-probes, rather than target DNA. A sensitive ligation-step ensures specificity of the assay. We designed MLPA-probes specific for a range of discriminatory SNPs in MTB. In addition, we selected three sequences of conserved regions to use as internal controls and for quantitation. The size of MLPA-products corresponds to the specific SNP they targeted.

Amplified MLPA-products were identified on an agarose-gel or by fragment analysis, using capillary electrophoresis.

Results: Probes were initially validated on DNA from reference strains. All probes were then combined in a single mixture and used to characterise laboratory strains and a panel of clinical isolates from Brazil, Peru and the Netherlands. To determine the predictive value of MTB-specific MLPA, we screened DNA of MTB-strains with unknown genotypes. Results were confirmed by sequencing.

There was a very high correlation between sequencing results and results obtained via MLPA. In addition, both the positive and the negative predictive value were high. MLPA-products were clearly visible on an agarose gel.

Conclusion: We have shown that with MTB-specific MLPA it is possible to identify drug resistance associated mutations and MTB lineage specific SNPs in a single assay. Depending on the application, probes can be added to or removed from the probe-mix, making it a flexible, multi-purpose method. MLPA products can be easily identified on an agarose-gel, making it especially suitable for screening of TB in the less-developed world.

Based on our results we feel that in addition to using MLPA on DNA extracted from culture, performing MLPA directly on DNA from sputum samples is also feasible.

O202 Differences in mutation rate between phylogenetic groups of *Escherichia coli*

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Objectives: The species *Escherichia coli* comprises four different phylogenetic groups (A, B1, B2 and D). In general the majority of infectious as well as colonising strains isolated from patients belong to phylogenetic group B2. Yet, there is a known disparity between fluoroquinolone-susceptible and -resistant isolates, which it is assumed has arisen from the susceptible population: Only a small proportion of resistant isolates belong to group B2, while the majority belong to group D. For a possible explanation of the disparate distribution among phylogenetic groups this work analysed ciprofloxacin-susceptible and -resistant ICU isolates in all phylogenetic groups for differences in virulence factors (VF) and mutation frequencies.

Methods: Hundred and forty-six susceptible and 135 resistant isolates were collected from patients in 27 ICUs. All strains were assigned to phylogenetic groups by means of a triplex PCR protocol. In order to compare isolates from different groups and susceptibilities, a similar and as large a number as possible of susceptible and resistant strains were chosen randomly from groups A (23; 27), B1 (7; 11), B2 (17; 9), and D (15; 25). The occurrence of different VF was determined by use of PCR and summarised for each isolate as a VF score. Moreover, the mutation frequency was determined by plating on 100 mg/L rifampicin containing agar.

Results: Assignation to phylogenetic groups confirmed differences between susceptible and resistant isolates in the distribution among phylogenetic groups: 82 out of 146 susceptible isolates belonged to group B2, in contrast to just 9 out of 135 resistant isolates, with a majority of 61 isolates belonging to group D. The VF score was highest both in strains belonging to groups B2 and D without significant differences between susceptible and resistant isolates. However, there was a small, but significant difference in the median of the mutation rates between the isolates of different groups, increasing in the order B2 < D < B1 < A. The median mutation rate of B2 isolates (1.3×10^{-8}) differed significantly ($P < 0.05$) from the remaining isolates as did, conversely, that of the group A isolates (2.4×10^{-8}).

Conclusion: Slightly, but significantly reduced mutation rates in strains of the deep rooted phylogenetic group B2 might be an explanation for their rare occurrence among fluoroquinolone-resistant isolates.

O203 Epidemiological surveillance and characterisation of TEM-, SHV-, and CTX-M-type ESBLs in Russian nosocomial strains of Enterobacteriaceae using real-time PCR and melting-curve analysis techniques

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Objectives: To determine the prevalence of major molecular class A extended-spectrum β-lactamases (ESBLs) of TEM, SHV and CTX-M families among nosocomial Enterobacteriaceae from Russian ICUs.

Methods: A total of 1373 consecutive nosocomial isolates of Enterobacteriaceae collected as part of the national surveillance study of antimicrobial resistance in ICUs of 32 Russian hospitals in 2002–2004 were screened for ESBL production using the MIC and double-disk synergy tests with cefotaxime, ceftazidime, cefepime and clavulanic acid. All strains with ESBL phenotype were tested for the presence of TEM, SHV and CTX-M ESBL-encoding genes using three real-time PCR assays. Two of these assays employed postamplification melting curve analysis with differentially labeled fluorogenic probes to detect all the known mutations associated with ESBL activity in blaTEM and blaSHV genes, respectively. The third assay targeting blaCTX-M genes utilised melting curve analysis with SYBR Green I to distinguish the members of CTX-M-1, -2, -8, and -9 clusters according to melting temperatures of PCR products generated with nested set of primers. Strains producing penicillinases SHV-1, TEM-1, SHV ESBLs with aa substitutions 146(V), 149(S), 156(D), 179(A,G,N), 238(A,S), 240(K), TEM ESBLs with aa substitutions 104(K), 164(C,H,S), 237(T), 238(S), 240(K) and CTX-M enzymes (–2, –3, –5, –8, –9, –14, –15) representing all four clusters were used as controls. Selected β-lactamase genes from isolates with unusual real-time PCR melting profiles were cloned and sequenced.

Results: ESBL phenotype was detected in 718 (52.3%) strains, members of 10 genera, most commonly in *Klebsiella pneumoniae* (81.4%), *Escherichia coli* (49.5%), *Citrobacter* spp. (59.3%) and *Proteus mirabilis* (38.9%). The distribution of various ESBL types and their combinations is summarised in the Table. In addition to the detection of known ESBL types, real-time PCR and sequencing allowed the identification of new SHV variants, each containing one of the following substitutions: 156D, 157E, 240K (alone), but lacking ESBL activity.

Conclusions: This study demonstrates the usefulness and efficiency of real-time PCR techniques for epidemiological typing of molecular class A ESBLs. It reveals the extremely high incidence of ESBLs, the dominance of CTX-M-1-cluster enzymes, frequent co-occurrence of CTX-M and SHV ESBLs, and surprisingly low incidence of TEM ESBLs among nosocomial Enterobacteriaceae isolated in Russian ICUs.

β-Lactamase types	Number	Percent in all ESBL producers
TEM (His164)	1	0.1
SHV-2-like (Ser238)	18	2.5
SHV-5-like (Ser238; Lys240)	78	10.9
CTX-M-1-cluster	431	60.0
CTX-M-2-cluster	1	0.1
CTX-M-9-cluster	28	3.9
CTX-M-1-cluster + SHV-2-like	44	6.1
CTX-M-1-cluster + SHV-5-like	98	13.6
CTX-M-2-cluster + SHV-2-like	3	0.4
CTX-M-2-cluster + SHV-5-like	1	0.1
CTX-M-9-cluster + SHV-5-like	8	1.1
Other ESBLs	7	1.0
Total TEMs/TEM ESBLs	373/1	51.9/0.1
Total SHVs/SHV ESBLs	425/250	59.3/34.8
Total CTX-M ESBLs	614	85.5

O204 Ceftazidime resistance evolution in ESBLs belonging to CTX-M-1 cluster (CTX-M-1, CTX-M-3, CTX-M-10) in a hypermutator background

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Objectives: Resistance to ceftazidime (CAZ) has mostly emerged among enzymes of CTX-M-1 lineage. The aim of this study was to understand evolution of CAZ resistance among CAZ susceptible ESBL belonging to this cluster by step-wise in vitro selection experiments.
Methods: blaCTX-M-1, blaCTX-M-3 and blaCTX-M-10 genes were cloned into pBGS18 plasmid vector (KanR) using EcoRI and PstI restriction enzymes. Recombinant plasmids were further transformed into *E. coli* strain MI1443 (del-ampC, plasmid free) and its hypermutable isogenic strain GB20 (mutS::Tn10 MI1443). GB20 transformants corresponding to each ESBL were submitted to daily serial passages with increased concentrations of CAZ (0.5–256 fold respect to MIC). Plasmids containing mutants obtained at distinct concentrations were transformed in MI1443 cells, selecting with CAZ (at 4× their corresponding MIC). MICs to CAZ, cefotaxime (CTX), cefuroxime (CXM), cefepime (FEP), and amoxicillin-clavulanate (AMX) were determined in MI1443 carrying the different evolved and non-evolved recombinant plasmids. blaCTX-M genes from strains containing recombinant plasmids displaying reduced susceptibility to CAZ were sequenced.
Results: A high frequency of CAZ mutants was detected (73%; 8/11 transformants tested) with significant increments in CAZ resistance. Interestingly, and with exception of two variants and CXM, a concomitant loss of resistance to all other antibiotics tested (antagonistic pleiotropy) was observed. All mutants contained new blaCTX-M variants except blaCTX-M-52, which evolved from blaCTX-M-3. The most frequent mutations found were D240G and P167S/T, the later conferring higher resistance to CAZ than the former. However, D240G change determined a lower decrease to FEP and CXM than P167S/T. Additionally, two variants were recovered from the evolved blaCTX-M-3 gene: P167S and P167S+A77V, found at 16 and 128 mg/L of CAZ respectively. The double mutant yielded higher resistance level to CAZ, CTX and to a lesser extent to FEP than P167S variant. The A77V change is one of the three polymorphisms between CTX-M-3 and CTX-M-1, whose mutants obtained revealed the highest resistance levels to CAZ and CTX in our study.
Conclusions: This work demonstrates an antagonistic relationship between the susceptibility to CAZ and to other β-lactam antibiotics tested. A77V could be a secondary mutation site affecting CAZ or could be involved in re-equilibrium of CTXR/CAZR co-resistance.

O205 Fluoroquinolone-resistant group A *Streptococcus* in Belgium: first report of an emergence of high-level FQ resistance in Emm6 GAS

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Objectives: Fluoroquinolone (FQ)-R group A *Streptococcus* (GAS) worldwide are predominantly of the emm6 serotype. Emm6 GAS are intrinsically low-level FQ-R due to a polymorphism in the FQ-binding region in parC, which confers resistance to the older FQs like ciprofloxacin (CIP). The new respiratory's FQs, levofloxacin (LEV) and moxifloxacin (MOX), retain their activity against these first-step parC mutants, although there are concerns that their widespread use might select for second-step, high-level FQ-R mutants.
LEV and MOX were introduced into clinical use in 1999 and 2002, respectively, in Belgium. We studied the prevalence and evolution of FQ-R GAS recovered from tonsillopharyngitis and invasive infections in Belgium during 2003–2005.
Methods: From a total of 3765 isolates collected from 10 provinces during 2003–05, 261 (7%) isolates were resistant to CIP (MIC $\geqslant$ 2 μg/mL) and corroborated with MICs of 4 FQs in the presence or absence of reserpine. Clonality was studied by pulsed-field gel electrophoresis (PFGE) and emm typing. Clonal isolates with CIP MIC $\geqslant$ 2 μg/mL were sequenced for mutations in the FQ binding regions of parC, gyrA, gyrB, and parE.
Results: During 2003–05, FQ-R GAS increased from 2%, 5% to 16%. The predominant emm type was emm6, which formed 76% of the total FQ-R GAS during these three years. Emm6 and emm75 isolates together constituted 92% of the FQ-R GAS. Isolates with CIP MICs 2–8 μg/mL showed previously identified mutations in parC leading to amino acid substitutions as S79A (emm6) and S79Yor S79F (emm75), and no changes in gyrA, gyrB or parE. However, an emm6 throat isolate, recovered from a 36-year old female in 2005, exhibited high-level resistance to CIP (MIC $\geqslant$32 μg/mL), full resistance to LEV (MIC 8 μg/mL) and decreased susceptibility to MOX (MIC 1 μg/mL). This isolate also showed a second-step mutation in the FQ-binding region in gyrA (predicted substitution S81Y). Reserpine-sensitive efflux was not observed for any FQ-R GAS.
Conclusions: We report a dramatic increase in FQ-R GAS in 2005 in Belgium probably related to natural fluctuations of the low-level FQ-R emm6 clone. More importantly, we describe, for the first time, an emergence of high-level FQ resistance in a clinical emm6 isolate. Although respiratory FQs are usually not a therapeutic option for GAS infections, dissemination of this parC/gyrA double-mutant emm6 strain needs close monitoring.

O206 Presence of family phage-like elements conferring efflux-mediated macrolide resistance in group G streptococci

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Objectives: Recently, we demonstrated that the prophage-like elements belonging to the Tn1207.3/Phi10394.4 family in *Streptococcus pyogenes* encode a DNA-modifying methyltransferase. In this study, the presence of the Tn1207.3/Phi10394.4 family was evaluated in group G streptococci (GGS) presenting the M phenotype.
Methods: Five GGS clinical isolates identified as *Streptococcus dysgalactiae* ssp. *equisimilis* and expressing the M phenotype of macrolide resistance were collected from 2002 to 2005 in Portugal. The presence of the mef gene as well as it linkage with the msr(D) gene, both involved in macrolide resistance, were investigated by PCR. The isolates were also characterised by PFGE. To identify the general structure of the mef genetic elements, the presence of phage-related ORFs belonging to the Tn1207.3/Phi10394.4 family was investigated by PCR, including the one encoding M.Spy10394I. Moreover, to discriminate between these two genetic elements, the structure of the left insertion site in the bacterial chromosome and a Phi10394.4-specific ORF, annotated as encoding an R28-like protein were investigated.

Results: The analysis of SmaI PFGE patterns showed that three of the isolates were clonally related while one was not typable by PFGE, since its DNA was resistant to SmaI digestion. All isolates showed the presence and linkage of mef and msr(D) genes and four carried the mef(E) variant while only one carried the mef(A) variant. Only the isolate, refractory to SmaI digestion and positive for the mef(A) variant, was also positive for all PCR reactions performed. The structure of the left insertion site of the Tn1207.3/Phi10394.4 family in the chromosome of this isolate revealed that in this streptococcal species the elements are inserted at the same locus as in *S. pyogenes*. Moreover, the absence of a PCR product for the ORF encoding the R28-like protein established Tn1207.3 as the element present in this strain.
Conclusion: The data demonstrated the presence of an element of the Tn1207.3/Phi10394.4 family in a GGS isolate. Resistance to SmaI digestion in this M phenotype isolate is probably due to the expression of the M.Spy10394I methylase, previously described in *S. pyogenes*. The dissemination of the Tn1207.3/Phi10394.4 family among *S. pyogenes* and GGS emphasize the important role of these related chimeric elements and their associated prophage in the worldwide emergence of macrolide resistant streptococci expressing the M phenotype.

O207 Plasmid-mediated quinolone resistance determinants, QnrA, QnrB, and QnrS, among clinical isolates of *Enterobacter cloacae* in a Taiwanese hospital

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Objectives: A total of 526 nonreplicate clinical isolates of *Enterobacter cloacae* collected in 2004 at a Taiwanese university hospital were analysed to: (i) determine the prevalence of three plasmid-mediated quinolone resistance determinants, QnrA, QnrB, and QnrS, and (ii) investigate the association of the Qnr determinants and the IMP-8 metallo-β-lactamase.
Methods: The qnrA-, qnrB-, and qnrS-like genes were detected by colony hybridisation and PCR-based experiments. Beta-lactamase contents of qnr-positive were determined by isoelectric focusing and PCR. Pulsed-field gel electrophoresis was used for strain typing. Restriction analysis of qnr-positive plasmids and blaIMP-8-positive plasmids were performed after conjugation experiments.
Results: Eighty-six (16.3%) of all isolates were qnr-positive, and the qnrA1-like, qnrB2-like, and qnrS1-like genes were detected alone or in combination in 3 (0.6%), 53 (10.1%), and 34 (6.5%) isolates, respectively. Among 149 putative extended-spectrum β-lactamase-producing isolates, 59 (39.6%) isolates, all of which were SHV-12 producers, harboured qnrA (0.7%, 1 isolate), qnrB (28.9%, 43 isolates), or qnrS (12.1%, 18 isolates). Forty-four (78.6%) of 56 IMP-8 producers carried qnrB (58.9%, 33 isolates), qnrS (25.0%, 14 isolates), or both. Conjugation experiments revealed coexistence of qnrB and blaIMP-8 on the transferred plasmids and the absence of β-lactamase content on the transferred qnrS-positive plasmids. The transferred blaIMP-8-positive plasmids with and without qnrB had very similar restriction patterns. Pulsed-field gel electrophoresis showed 6 major patterns among the 44 qnr-positive IMP-8-producing isolates.
Conclusion: This study demonstrated the high prevalence of qnr among *E. cloacae* isolates at a Taiwanese hospital. The extreme high prevalence of qnr among metallo-β-lactamase-producing *E. cloacae* isolates may be mainly due to intra-hospital spread of several clones and dissemination of plasmids containing both qnrB and blaIMP-8.

O208 Biological fitness of fluoroquinolone-resistant invasive clinical isolates of *Streptococcus pneumoniae*

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Background: Resistance to fluoroquinolones in pneumococci is emerging globally. Biological fitness of resistant strains in absence of selective pressure is an important parameter determining the spread and persistence of resistance. In vitro studies using isogenic isolates have shown that most of the mutations in the quinolone resistance determining regions are associated with a fitness cost. In this study resistant clinical isolates (LFX $\geqslant$ 8 mg/L) were used to take into account potential compensatory mutations.
Methods: Invasive isolates related to international multiresistant clones defined by the Pneumococcal Molecular Epidemiology Network (PMEN) were analysed (Antimicrob Agents Chemother 2004; 48: 3491–7). Because isogenic susceptible ancestors of resistant clinical strains can not be obtained, the isolates studied were compared with their respective fluoroquinolone-susceptible PMEN reference clone.
Growth curves were determined in three independent experiments with three replicates each by measuring optical density at 600 nm. Relative fitness was assessed comparing the slope of the curve of each isolate with its respective reference clone by Mann–Whitney U test.
Results: Five clusters of isolates (Spain 23F-1: n = 8; Spain 9V-3: n = 5; England 14–9: n = 5; Greece 6B-22: n = 5; Utah 35B-24: n = 3) were examined. For the majority of isolates no significant fitness cost was found when compared to their respective reference clone. In three clusters (Spain 9V-3, England 14–9, Greece 6B-22) individual isolates with a fitness gain could be identified. Only in the Spain 23F-1 cluster, 3 of 8 isolates exhibited a fitness cost which was associated with a serotype switching from 23F to 19F.
Conclusion: We found notably few resistant clinical isolates with a fitness cost. Therefore even in the absence of selective pressure (fluoroquinolone usage), fluoroquinolone-resistant strains may not be replaced completely by susceptible strains.

O209 Large scale spa-typing of *Staphylococcus aureus*: a reference centre's view

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Objectives: In May 2006 we introduced spa-typing in our laboratory. This method replaced phage typing in combination with SmaI-macrorestriction analysis for routine *S. aureus* strain typing. This study reviews typing results for six month with respect to typeability, reproducibility, discriminatory power and concordance with alternative typing methods.
Methods: A total of 1464 *S. aureus* isolates were characterised. The polymorphic X-region of the protein A gene was sequenced and a spa type was assigned using the Ridom StaphType software. The algorithm BURP (based upon repeat patterns) was used to cluster resulting spa types into different groups. To verify the results a subset of isolates was subjected to SmaI-macrorestriction analysis and MLST.
Results: Among 1464 *S. aureus* isolates more than 200 different spa-types were defined. The twenty spa-types most frequently found comprise more than 60% of all isolates and include most types linked to predominant clonal lineages of MRSA in Central Europe, previously defined by phage typing and SmaI-macrorestriction analysis. Among those clonal lineages distinct differences in spa-type variability were seen; while some lineages (e. g. the Barnim epidemic MRSA, t032, ST22) encompassed a variety of closely related spa-types, others (e. g. a subclone of Rhine-Hesse epidemic MRSA, t003, ST225) are represented only by a single type. For assessment of widely disseminated lineages exhibiting only a single spa-type, additional markers must be applied.
More than one hundred spa-types were determined only once. For these isolates BURP proved to be a valuable tool to assign them to particular clonal groups. Thereby, results of BURP grouping were overall concordant with those of SmaI-macrorestriction analysis and MLST.
Conclusion: spa-typing in combination with BURP based grouping of isolates proved to be a valuable tool for strain typing with regard to the demands of a national reference centre. The BURP algorithm revealed extremely useful for grouping new and uncommon spa-types. The increasing amount of typing data obtained from outbreak situations enables comparison of closely related spa-types within BURP groups, thus facilitating the definition of criteria for strain relatedness according to those already defined for SmaI-macrorestriction analysis. This will be an important prerequisite for definition of isolates as identical, related of different, which is essential for successful epidemiological outbreak investigation.

Novel insights in preventing paediatric bacterial respiratory diseases beyond pneumococci (Symposium organised by GSK)

S218 Childhood bacterial respiratory tract infections in the conjugate vaccination era

R. Dagan (Beer-Sheva, IL)

Although bacterial colonisation is common and mostly asymptomatic in children, it frequently results in a wide spectrum of respiratory tract infections (RTIs): acute otitis media (AOM), sinusitis, conjunctivitis, pneumonia and other lower respiratory infections (LRIs). In children, the majority of bacterial RTIs are caused by *Streptococcus pneumoniae* (Pnc), *Haemophilus influenzae* (mostly nontypeable, NTHi) or both. In AOM, NTHi and Pnc are equally common. Pnc is slightly more 'aggressive' than NTHi (i.e. higher temperature, higher polymorphonuclear counts and complications are more frequent), but NTHi can still cause significant disease. The role of NTHi in recurrent and non-responsive AOM in both infants and in older children is recognized, suggesting different biological pathways compared with Pnc. In both acute and subacute sinusitis, NTHi is found as commonly as Pnc. NTHi is the most common causative agent of conjunctivitis and the most significant in relation to the co-presence of AOM and systemic symptoms and signs. The role of NTHi in LRIs in general, and in pneumonia in particular, is not yet clear. In cystic fibrosis, however, NTHi is an important pathogen. Although *H. influenzae* type b disease has practically been eliminated in vaccinated populations, the picture with *S. pneumoniae* in the 7-valent Pneumococcal Conjugate Vaccine (PCV7) era is much more complex owing to its limited serotype coverage and some replacement with non-vaccine serotypes. A vaccine with additional pneumococcal serotypes and activity against NTHi would be a very welcome addition to our vaccine armamentarium.

S219 The role of bacterial biofilms in chronic and recurrent otitis media

L.O. Bakaletz (Columbus, US)

Otitis media (OM) is a highly prevalent paediatric disease that is associated with significant morbidity and socioeconomic cost. OM is caused by the synergistic interaction between upper respiratory tract viruses and three predominant bacterial species, all of which are members of the commensal flora of the paediatric nasopharynx. Despite their similar roles as opportunistic pathogens of the middle ear, the pathogenic mechanisms utilised by these bacteria (nontypeable *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Moraxella catarrhalis*) are unique. Thus, to date, most laboratories have investigated the pathogenesis and prevention of disease caused by these microbes individually. Recently, it has become appreciated that OM, and particularly chronic and recurrent OM, is a disease that includes a biofilm component. By definition, bacteria resident within a biofilm community are inherently resistant to eradication due to their significantly enhanced ability to resist the action of antibodies as well as antibiotics. Moreover, residence within a biofilm promotes the bacterial exchange of DNA, which confers resistance to antibiotics, and induces a markedly reduced bacterial growth rate that commonly results in false-negative bacterial cultures.

In the past few years it has been demonstrated that each of the three predominant bacteria involved in OM can form a biofilm in in vitro assay systems and in animal models, and that they participate in biofilms formed on middle ear mucosa specimens recovered from children with recurrent or chronic OM. This enhanced understanding of the biofilm component of middle ear infections is greatly influencing the current approaches to both the management of OM and the design of novel strategies to treat, or better to prevent, OM. It is particularly intriguing that some of the paediatric middle ear mucosa-derived biofilms characterised were of mixed bacterial aetiology, suggesting that progress made on single-microbe directed strategies for the treatment and/or prevention of OM, while highly encouraging, are also likely to be inadequate. Therefore, a significantly greater understanding of how microbial physiology relates to the involvement of biofilms in OM is required to identify points in the disease course that are perhaps more amenable to treatment strategies, and also biofilm-relevant antigenic targets that would be helpful in the rational design of vaccine candidates to prevent OM.

S220 Protein D of *Haemophilus influenzae* – the antigenic potential of a carrier protein

A. Forsgren, K. Riesbeck, H. Janson (Malmö, SE)

Haemophilus influenzae (Hi) is a Gram-negative cocco-bacillus, which is a common commensal in the nasopharnyx of healthy children. Hi exists in encapsulated and non-encapsulated forms based on the expression of capsular polysaccharide proteins. Six encapsulated strains are recognized (a, b, c, d, e and f), while non-encapsulated strains are considered non-typeable (referred to as NTHi).

Protein D (PD) is a highly conserved surface exposed 42 kDa lipoprotein found in 100% of all NTHi and encapsulated Hi, and is highly immunogenic in humans. Evidence suggests that PD is involved in the pathogenesis of respiratory tract infections, and has been implicated in NTHi adhesion and invasion of host cells.

An isogenic PD-deficient NTHi-mutant, compared with the wild type strain, had an approximately 100-fold lower capacity to cause acute otitis media (OM) in rats and induced significantly less impairment of ciliary function in a human nasopharyngeal tissue culture model. The likely mechanism of pathogenicity is that PD is an enzyme (glycerophosphodiester phosphodiesterase, GlpQ) that releases phosphorylcholine (ChoP) from host epithelial cells to the lipooligosaccharide (LOS) on NTHi. LOS-ChoP interacts with platelet-activating factor receptors, inhibiting ciliary beat frequency of human bronchial epithelial cells and activating G protein-related pathways.

PD is also capable of inducing bactericidal anti-protein D antibodies that protect against NTHi disease. PD-immunised rats cleared NTHi significantly better than non-immunised animals after middle ear and pulmonary bacterial challenge. In addition, chinchillas, PD-immunised or passively given anti-PD sera, showed protection against NTHi-dependent OM.

PD was recently introduced as an antigenically active carrier protein in an experimental 11-valent pneumococcal conjugate vaccine. In a recent Phase III clinical trial with this experimental vaccine, significant protection was achieved against OM caused by pneumococci and/or NTHi.

S221 The added value of measuring function of antibodies in conjugate vaccine trials

H. Käyhty (Helsinki, FI)

The currently licensed pneumococcal conjugate vaccine (PCV) has been integrated into infant immunisation programmes of several industrialised countries. New PCV candidates in development with wider serotype coverage or new PCV formulations for both infants and adults will be evaluated for efficacy. Large efficacy trials are not feasible any more, and the acceptance of the new PCVs will rely on immunogenicity studies. In the WHO guidelines (http://www.who.int/biologicals/publications/trs/areas/vaccines/pneumo/en/index.html) the primary threshold for such trials is the proportion of subjects with antibody concentration of $\geqslant 0.35\,\mu g/mL$ for all serotypes when measured via the WHO non-22F ELISA. The protective functional mechanism of antibodies to pneumococcal capsular polysaccharides resides in opsonophagocytosis and the demonstration of the opsonophagocytic activity (OPA) has been regarded as an important secondary threshold.

Thus far there are only few published PCV efficacy trials that connect with OPA data and their good correlation. Considering those data it is becoming evident that measuring OPA has an added value when

evaluating the vaccine efficacy based on immunogenicity studies. In general, the OPA titers and the antibody concentrations correlate well, but different serotypes can show various OPA/EIA ratios meaning that for certain serotypes (like 19F or 1) more antibodies are needed for killing the bacteria than for the rest. Importantly, the elderly and HIV positive patients seem to have antibodies of low functional activity, and measuring OPA in studies in the elderly and HIV patients has been considered important.
Various assays for determining OPA have been developed and some laboratories have already performed extensive validation steps. A multilaboratory standardisation of the OPA assay is imminent. Measuring OPA with the classical killing assay consumes serum and time and thus measuring OPA for large materials is not always feasible. However, the development of multiplex and/or high throughput assays will end up in wider use of this technology in the future clinical trials. This would be in concordance with the guidelines for evaluation meningococcal conjugate vaccines by serum bactericidal activity assay.

S222 A new protein conjugate vaccine for polymicrobial diseases: a case study (POET)

W.P. Hausdorff (Rixensart, BE)

Bacterial respiratory tract infections are a leading cause of morbidity and mortality in young children. Effective immunisation strategies against these infections have to contend with both the multiplicity of pathogens and a host of disease syndromes. *Streptococcus pneumoniae* (pneumococcus), and both encapsulated and non-encapsulated forms of *Haemophilus influenzae* (Hi), are the leading pathogens. More than 90 serotypes of pneumococci exist, but only 10 serogroups appear to be responsible for 80–85% of invasive pneumococcal disease and pneumococcal otitis media (OM) worldwide, ranging from the relatively rare but clinically severe meningitis, to the more frequent infections of the bloodstream and the lungs, such as pneumonia and finally to the extremely common middle ear infections among young children.
Middle ear infections are often associated with diagnostic uncertainty, frequent use and misuse of antibiotics, and recurrent and persistent disease. Recurrent infections occur in as many as 10% of children <3 years of age. Recurrent and persistence disease can lead to temporary or even permanent hearing loss and surgery (tube placement). In order to address these issues, preventive acute OM (AOM) strategies should include a vaccine with broader pneumococcal serotype coverage as well as protection against additional otopathogens.
This presentation describes a novel vaccine approach in which polysaccharides from the 11 leading streptococcal paediatric serotypes are conjugated to a carrier protein (protein D) derived from Hi. In the Pneumococcal Otitis Media Efficacy Trial (POET), conducted in infants and young children in the Czech and Slovak Republics, this approach resulted in substantial protection against pneumococcal and Hi diseases. In this study, an 11-valent investigational conjugate vaccine was concomitantly administered with diphtheria, tetanus, pertussis (DTP) vaccines. The clinical diagnosis of AOM was confirmed by both a paediatrician and an otorhinolaryngologist, and tympanocentesis was performed on children with confirmed AOM. Vaccine efficacy against any AOM episode caused by vaccine-type pneumococci was 57.6% (95% CI: 41.4–69.3%), and efficacy against AOM caused by nontypeable Hi was 35.3% (1.8–57.5%). Most strikingly, the overall impact of the vaccine in this setting was to reduce all AOM by 33.6% (20.8–44.3%). These results suggest that a vaccine that targets these two major groups of bacterial pathogens would have a significant public health impact.

Staphylococcal disease: from nose to morgue

S237 Staphylococcal components required for host–pathogen interaction as vaccine targets

S. Foster (Sheffield, UK)

The threat of multidrug resistant *Staphylococcus aureus* has renewed efforts to develop a prophylactic vaccine and therapeutic/prophylactic antibodies to combat this serious pathogen. A number of potential vaccine components have been investigated including capsule, a surface polysaccharide polymer and several surface proteins. However nothing is available in the clinic at the moment. The search for vaccine components has highlighted a number of proteins with important roles in host:pathogen interaction. These include a family of proteins specifically produced under conditions of iron limitation. A prominent member of this family (IsdA) is required for nasal colonisation and vaccination with IsdA prevents carriage.
The current status of vaccine development and how this has led to interesting insights into interaction between *S. aureus* and its host will be reviewed.

Presence and future of *Clostridium difficile*

S238 *Clostridium difficile* in Europe: results of the 2005 European-wide survey

F. Barbut, P. Mastrantonio, M. Delmée, G. Ackermann, E. Bouza, C. Balmelli, D. Drudy, E. Kuijper, H. Ladas, E. Nagy, H. Pituch, M. Wullt, M. Yücesoy, M. Rupnik, I. Poxton for the European Study Group on Clostridium difficile (ESGCD)

The recent emergence of the epidemic *Clostridium difficile* strain 027 in Europe could lead to important changes in the epidemiology of *C. difficile*-associated diseases (CDAD) and requires an ongoing clinical and molecular surveillance of CDAD.
A two-month prospective study on *C. difficile* was conducted in 38 hospitals from 14 different European countries in order to get an overview on the phenotypic and genotypic features of isolates in 2005. Strains were isolated from diarrheic patients with suspected CDAD. They were characterised by toxinotyping and production of toxins A and B. Binary toxin genes (cdtA and cdtB) were detected by PCR. Minimal inhibition concentrations (MIC) of metronidazole, vancomycin, erythromycin, clindamycin, moxifloxacin and tetracycline were determined using the Etest method. Epidemic 027 strain was identified by PCR-ribotyping. Of 406 strains, 349 were toxigenic of which 89 (25.5%) were toxin variant strains. Major toxinotypes included toxinotype 0 (n = 260), V (n = 27), VIII (n = 22) and III (n = 22). Resistance to erythromycin, clindamycin, moxifloxacin and tetracycline was found in 45.8%, 50.6%, 9.1% and 33.7%, respectively. All the strains were fully susceptible to metronidazole and vancomycin. Resistance to moxifloxacin and erythromycin was tightly associated and MICs of moxifloxacin were significantly higher in patients previously treated with fluoroquinolones. Prevalence of the 027 epidemic strain was 5.7%. It was found in Ireland (n = 1), Netherlands (n = 8) and Belgium (n = 11). This strain exhibits the same phenotypic and genotypic features as the NAP1 clone described in North America: it is positive for binary toxin genes, it has an18-bp deletion in tcdC gene and it is resistant to erythromycin and moxifloxacin. Mean incidence of CDAD was 2.45 cases per 10,000 patient-days but it varied widely from one hospital to another. Patients infected with 027 strain were likely to have a more severe disease (RR = 1.72, 95% CI: 1.07–2.75, p = 0.048), to have received less antimicrobials in the month preceding diarrhoea (RR = 0.76, 95% CI: 0.53–1.10, p = 0.05) and to have been more specifically treated by metronidazole or vancomycin (RR = 1.32, 1.17–1.49, p = 0.02). On-going epidemiologic surveillance of CDAD cases with periodic characterisation of the strains is needed to timely detect clustering of cases and to monitor the emergence of a specific hypervirulent clone.

S239 Novel insights into the molecular diagnostics and epidemiology of *C. difficile*

M. Rupnik (Maribor, SI)

The incidence and severity of nosocomial *C. difficile* disease seems to be increasing. Additionally, disease with community onset is becoming more common than previously recognized and cases in groups with

previously low risk have been reported. *C. difficile* is also emerging as an important animal pathogen in horses, calves and piglets.
Described changes correlate with changed populations of *C. difficile* present in humans. New type BI/NAP1/027 with increased virulence is spreading since 2003, but only part of the recent increase in mortality and morbidity caused by *C. difficile* infections can be accounted for by this new highly virulent type. The proportion of other binary toxin positive types is also rising among human isolates. Such binary toxin positive strains used to be more often associated with animal than human host and early typing comparisons did not show many similarities between human and animal strains. However, recent studies found a marked overlap between isolates from calves and humans, including two of the predominant outbreak types, 027 and 017. *C. difficile* has also been found in retail meat samples, suggesting that food could be involved in the transmission of *C. difficile* from animals to humans.
As a response to the new developments in *C. difficile* epidemiology, new diagnostic and typing methods are being developed aiming in quick and sensitive detection of *C. difficile* but also in quick recognition of strains with higher virulence.

S240 Special challenges in *C. difficile* infection control

E. Kuijper, R. Vonberg, M. Wilcox, P. Gastmeier (Leiden, NL; Leeds, UK; Hannover, DE)

Clostridium difficile is an anaerobic bacterium capable of forming spores which confer resistance to heating, drying and chemical agents, including disinfectants. Spores of CD may survive in the environment for long periods and are resistant to alcohol. More than 150 PCR ribotypes and 24 toxinotypes have been recognized; epidemic ribotypes may have enhanced sporulation. The spectrum of *C. difficile*-associated disease ranges from asymptomatic carriage to fulminant, relapsing and potentially fatal colitis. Since 2003, increasing rates of CDAD have been reported in North America and Europe involving a more severe course, higher mortality, increased risk of relapse and more complications. The outbreaks are difficult to control and require a multifaceted approach. The most important infection control measures act on interruption of transmission of spores to vulnerable patients from infected patients and from the environment. Vulnerable patients are mainly patients who receive antimicrobial treatment, and therefore fewer antibiotic prescriptions should lead to less vulnerable patients. At present, no sufficient evidence exists to propagate the use of probiotics to vulnerable patients for prevention of CDAD. Transmission of spores occurs mainly via contact of contaminated healthcare workers to patients, directly by patient-to-patient transmission or by transmission from the contaminated environment to patients. There is no evidence that patients or healthcare workers who are symptom-free but colonised with *C. difficile* in the intestinal tract are significant sources of infection. Airborne transmission of infection is unlikely to occur based on recent reviews of the literature. Early diagnosis of CDAD, prompt isolation of symptomatic patients and reducing antimicrobial treatment are essential first steps. The infection control measures include recommendations to isolate infected patient on a single room with designated toilet, to apply proper hand hygiene with soap and water, to use appropriate protective clothing (gloves and aprons or gowns), to intensify environmental cleaning with a chlorine containing disinfectant and to take specific precautions for the use of devices (disposable or dedicated to individual patient). Patient isolation must continue at least until diarrhoea has ceased. Each hospital should have an appropriate surveillance system to recognize an increase of the incidence of CDAD in an early stage. All infection control measures should be written in a local protocol so that additional measures can be carried out as soon as a problem with CDAD arises. When outbreaks occur, additional recommendations include a reinforcement of general and hand washing measures, intensifying of testing patients with diarrhoea for *C. difficile*, reinforcement of environmental cleaning, information and education of healthcare workers, cleaning department and visitors, cohorting of infected patients, and eventually closure of the unit followed by intensive environmental cleaning. Restricted antibiotic prescribing is also highly recommended to reduce polypharmacy and duration of administration. Second and third generations cephalosporins, clindamycin and more recently fluoroquinolones have been identified as potential risk factors. Although some hospitals report successes for enhanced environmental cleaning with potentially effective agents such as hydrogen peroxide vapour, the evidence is too scarce to consider this as an evidence-based approach.

Acinetobacter – the Gram-negative MRSA?

S242 Global epidemiology of *Acinetobacter*

K.J. Towner (Nottingham, UK)

Members of the genus *Acinetobacter*, particularly *Acinetobacter baumannii*, are now recognized as significant nosocomial pathogens, particularly for the subset of critically-ill patients requiring mechanical ventilation in hospital intensive care units. However, *Acinetobacter* infections can also be acquired outside the healthcare setting. Wound infections caused by *Acinetobacter* are now common among trauma patients in conflict zones or following natural disasters, and there have also been reports of other community-acquired infections, mainly in patients with some type of co-morbidity. Most of the latter reports originate from tropical or sub-tropical areas, and may become more common following ongoing changes in the global climate.
Strains of *A. baumannii* harbour inherent or acquired resistance mechanisms against the vast majority of available antimicrobial agents, including the carbapenems. These organisms also have a remarkable capacity to persist in the hospital environment and to cause epidemic outbreaks of infection in hospitals. A recent increase in the number of carbapenem-resistant strains, particularly in eastern Europe, is currently of great concern. The recent EU-funded ARPAC study revealed that 130 of 169 participating European hospitals in 32 European countries had encountered carbapenem-resistant isolates of *Acinetobacter*, ranging from very rare sporadic resistant isolates to an endemic/epidemic situation. Certain multiresistant epidemic clones are currently spreading worldwide, and epidemics of multiresistant *A. baumannii* infections have occurred following the repatriation of casualties from the recent wars in Kuwait, Iraq and Afghanistan to European and American hospitals.
In general, multiple isolates from a single hospital usually belong to the same clone, but some hospitals yield isolates belonging to more than one clone. Three 'European Clones' (lineages) have been identified in hospitals throughout Europe, but numerous other, more localised, clones have also been characterised, showing that the problem of *A. baumannii* is not confined solely to the widespread 'European clones I, II and III'. The reasons why certain *A. baumannii* lineages are more successful than others are not understood at present.
Overall, the available epidemiological evidence suggests that *A. baumannii* is becoming one of the most significant microbial challenges of the current era. Strains in some hospitals are already effectively untreatable, and more scientific efforts and resources are urgently needed to further elucidate the epidemiological and infection control issues related to these infections.

S243 Current resistance trends and mechanisms in *Acinetobacter baumannii*

P. Nordmann (Paris, FR)

Acinetobacter baumannii is an opportunistic pathogen that is mostly involved in nosocomial infections in immunocompromised patients. Whereas *A. baumannii* has itself a quite high level of naturally-occurring antibiotic resistance, it may acquire additional resistance traits as a source of multidrug resistance. These resistance mechanisms may involve most of the antibiotic molecules, i.e. aminoglycosides, β-lactams and fluoroquinolones. Rate and spread of specific mechanisms of resistance depends on each country. A similar trend between the number of *A. baumannii* infections and antibiotic resistance may be observed worldwide. Acquired resistance genes are located on chromosome, plasmids, insertions sequences, transposons, integrons,

and the recently described resistance island. One of the most worrying antibiotic resistance problems in *A. baumannii* is the increasing trend of carbapenem resistance since carbapenems are often used as antibiotics of the last resort. Carbapenem resistance results from metallo-β-lactamases, carbapenem-hydrolyzing oxacillinases and often combined mechanisms of resistance.

Emerging infectious diseases

O246 Risk assessment and management of possible transmission of Lassa virus during two flights

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Objectives: Since the emergence of Lassa fever, reports of about 25 patients who imported Lassa virus to Europe, USA or Canada have been published. More than 1,200 contacts were ascertained in these events and all remained pathogen free except one who showed a seroconversion. On July 10th a person (patient) who flew from Freetown (Sierra Leone) to Frankfurt (Germany) via Brussels (Belgium) tested Lassa positive nine days after arrival in Germany. A risk assessment was conducted to guide the decision on a co-passenger trace back.
Methods: During the two flights the patient had no cough but a urinary tract catheter which was disconnected from the reservoir and released relevant amounts of urine on the passenger's seat. The patient's urine was tested positive for Lassa virus, which was taken into account for the risk assessment and contributed to the decision to trace back co-passengers potentially exposed.
The passengers at risk were defined as those sitting in maximum three rows distance from the patient. 57 additional persons from the airline and the airport were exposed.
With the help of the airline the passenger's lists of both flights were available within two days. 92 passengers from nine countries were identified. Every country received a list with names and contact telephone numbers of all passengers at risk who were allocated to the country as well as a questionnaire about symptoms to be filled for traced passengers. The trace back of the concerned staff was done by the airline and the Belgian public health authorities.
Results: In EU countries 29 (66%) of 44 contact passengers, in European non-EU countries 7 (68%) of 9 passengers were traceable and from non-European countries 0 of 27 passengers were traceable. 100% of the staff were traceable. Overall 62.4% of the contacts were traceable. Only one of the traced contacts developed symptoms but revealed to be Lassa negative.
Conclusion: In this investigation, the leakage from the urinary catheter reservoir influenced the risk assessment and the decision for a passenger trace back as Lassa fever is primarily transmitted by urine of rats. Nevertheless, no contact passenger revealed Lassa virus. Our investigation confirms that the human to human transmission of Lassa virus during flights is unlikely if no haemorrhagic symptoms appear at this time. Tracing back co-passengers remains a challenge internationally and stresses the need for an early voluntary implementation of the revised international health regulation.

O247 Comparison of oral ribavirin treatment in Crimean–Congo haemorrhagic fever: a historical cohort study in Turkey

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Objectives: To analyse the efficacy of oral ribavirin treatment in Crimean–Congo haemorrhagic fever (CCHF) patients and to compare with a historical cohort.
Methods: In Turkey, patients admitted to four tertiary care hospitals with a disease resembling CCHF were treated with oral ribavirin as recommended by the World Health Organisation (WHO) between April and September, 2004. Treated patients were compared with an untreated historical cohort who admitted to the same hospitals in 2003. Sera from suspected CCHF patients were obtained immediately following hospitalisation. Whenever possible, a second sample was obtained at least one week later. Serologic and virologic analyses were performed in the CCHF reference laboratory of the RSH Institute of the Turkish Ministry of Health. Only the patients that obtained a definitive diagnosis of CCHF by means of clinical presentation and the presence of specific IgM antibody against CCHF virus and/or viral RNA were included in the study. Demographics of all patients, clinical and laboratory findings, given blood and blood products, length of hospitalisation stay and outcome were recorded.
Results: The treatment group and the historical cohort consisted of 126 and 92 confirmed CCHF cases respectively. The mean age of the treatment group was 44 and 41 years in the historical cohort ($p > 0.05$). Among the given mean units of blood products, only the amount of consumed fresh frozen plasma was significantly lower than the treatment group (median 4 vs 6.5 units; $p < 0.05$). Median length of hospitalisation days was 8 in the treatment group and 9 days in the historical cohort ($p > 0.05$). The case fatality rate in the treatment group was not significantly different than in the historical cohort (7.1% vs 11.9%; $p > 0.05$). A logistic regression analyse showed altered sensorium and/or prolonged international normalised ratio (>1.4) were independent predictors of mortality. These predictors discriminated fatal cases with a sensitivity of 0.73 (14 of 19 fatal patients) and a specificity of 0.83 (156 of 186 non-fatal patients).
Conclusions: The results of this study showed that oral ribavirin treatment failed to improve the survival rate in our confirmed CCHF cases. However, more controlled studies with oral ribavirin are needed before more definite conclusion. We suggest the use of only the parenteral form of ribavirin according to a risk assessment by the predictors of mortality.

O248 Crimean–Congo haemorrhagic fever among children in Southeast Iran (clinico-epidemiological feature and outcome analysis)

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Background: Crimean–Congo haemorrhagic fever (CCHF) is a viral haemorrhagic illness of the nairovirus group. Althought primarily it is a zoonosis, sporadic cases and outbreaks of CCHF affecting humans do occur. The disease is endemic in many countries in Africa, Eastern Europe and Asia. During recent years, outbreaks have been reported in South Africa, the Middle East and Iran. Despite the endemicity of the disease especially in the Southeast of Iran, data on CCHF in children from Iran are limited. This study was conducted to detect the risk factor and clinico-epidemiological feature and outcome analysis regarding efficacy of ribavirin in children with CCHF in Iran.
Patients and Method: Between 1999 and 2006, the study included 34 cases under the age of 18 years who were admitted to BooAli hospital in Systan province of Iran. The diagnosis was confirmed through detection of IgM ELISA and/or genomic segment of PCR CCHFvirus.
Results: Out of 34 children with Crimean–Congo haemorrhagic fever (23 male, 11 female) with age range of 5 to 18 years, 29 patients (85%) were from rural areas and tick bite was determined as a risk factor for 23.5% of affected children. The most observed symptoms were fever (85.2%), myalgia (67.6%) and bleeding (61.7%). The most common sites of bleeding were nasal and oropharyngeal mucosa, with gastrointestinal tract ranking second. High fever (>38.5ºC) during hospitalisation, confusion, bleeding from multiple sites, and presence of petechia/echymosis occurred more often in those patients who died than in surviving ones. (The presence of high fever during admission, confusion, bleeding from multiple sites, and petechia/echymosis were associated with higher mortality rate in admitted patients.) Additionally, the mean values of ALT, AST, PTT, INR and urea were also higher, and mean platelet count was lower in the patients who died. Nearly all of the patients (except two) were treated with ribavirin. The recovery rate was

higher in children whose treatment started during the initial 3 days of illness in comparison to children whose treatment started after the first 3 days (85.2% versus 24.8%).
Conclusion: In children who suffered from CCHF in southeast of Iran, clinical features, factors influencing outcome of disease and risk factors were similar to other outbreaks of this disease in adult patients in Iran. Treatment with oral ribavirin can increase recovery rate in children as well as adult patients.

O249 Clinical features of dengue infections, and predictors of dengue haemorrhagic fever in Singapore

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Objectives: In 2004, Singapore experienced its worst dengue outbreak in 30 years with 9,459 notified cases, of which 80% were hospitalised. The study objective was to explore the demographic, clinical and laboratory features of dengue fever (DF) and dengue haemorrhagic fever (DHF) upon presentation to the hospital, and to determine predictors of DHF as a marker of severity.
Methods: A retrospective study was conducted on all laboratory-diagnosed dengue cases admitted from 1 Jan to 31 Dec 2004 to Tan Tock Seng Hospital (TTSH), the main dengue treatment hospital in Singapore. Demographic, clinical, and laboratory data were collected from the hospital's emergency department and standardised dengue clinical pathway during admission. Using data throughout the admission, cases were classified as DF or DHF according to the World Health Organisation classification. Data on presentation was then used to determine the predictors of subsequent development of DHF.
Results: The study included 2,144 laboratory-diagnosed dengue cases. Of these, 140 (6.5%) were classified as DHF, with one death. On presentation, the mean age among DF and DHF patients were not significantly different (35.1 years compared to 32.3 years respectively). However, there were more male DHF patients (74.3%) compared to DF patients (63.2%, $p < 0.01$). DHF patients had more frequent dehydration on admission (20.7%), compared to DF patients (2.9%, $p < 0.01$). DHF patients also had more co-morbid medical conditions (27.9%) compared to DF patients (10.4%, $p < 0.001$). For the laboratory results, DHF patients had lower mean white cell count (2.99×10^3/uL) compared to DF patients (3.65×10^3/uL, $p < 0.01$), and lower total protein levels (2.95 g/dL) compared to DF patients (6.32 g/dL, $p < 0.01$). Mean prothrombin and partial thromboplastin times, and atypical lymphocytes counts were also significantly lower in DHF compared to DF patients. From the multivariate analysis, males [odds ratio (OR) = 1.61], dehydration (OR = 6.06), white cell count (OR = 0.86), total protein levels (OR = 0.96), and atypical lymphocyte counts (OR = 0.95) were independently and significantly associated with DHF.
Conclusions: A few key routine demographic, clinical, and laboratory variables collected on admission may be used to predict DHF. These variables can be used by clinicians to determine the likelihood of DHF occurring during the admission, and may also be used as a marker to determine the need for admission or close monitoring.

O250 Transmission potential of Chikungunya fever in a two-wave epidemic in Reunion Island

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Background: A Chikungunya fever epidemic, transmitted by *Aedes albopictus* mosquitoes, has swept Reunion Island (Indian Ocean), causing 3000 cases in a first wave (April–May 2005) then 260,000 cases in a second wave (December 2005–May 2006). The reason for this dramatic increase between the two seasons is unknown, but it has been suggested that molecular changes may have increased the transmission potential.
Methods: We assessed the transmission potential through determining the reproduction number R (i.e. average number of secondary cases per index case) during the course of the epidemic. R values larger than 1 correspond to an epidemic outbreak. The method uses the epidemic curve and the generation interval distribution (time from symptoms in the index case to symptoms in a secondary case). The generation interval distribution was reconstructed by composing the latent, viremic and incubation period in humans, as well as the bite rate and mortality in mosquitoes.
Results: R was larger than 1 during 6 weeks in the 2005 epidemic (average 2.3), and during 20 weeks in the 2005/2006 epidemic (average 1.7). In all cases, the magnitude of the reproduction number was comparable between the two waves. Our best estimate for the initial reproduction number (R_0) was 3.7, although it could range from 2 to 11 depending on assumptions regarding incubation and lifespan in mosquitoes. In the best fitting case, each infected individual may have contaminated 3 mosquitoes at most, and each mosquito contaminated 1.4 persons on average. Using data from the first season alone, model-based extrapolations suggested that epidemic outbreaks were possible as long as more than one third of the population remained susceptible.
Conclusion: Despite a thousand-fold change in incidence between the two seasons, the transmission characteristics of Chikungunya were similar; therefore there is no epidemiological evidence for an increase in virulence between seasons. At a time when information systems make it easier to monitor the course of emerging diseases, methods for timely and efficient analysis of the data must also be developed.

O251 Seroprevalence and reservoirs of leptospirosis in Conakry (Guinea)

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Objectives: From September to December 2001, an urban outbreak of febrile jaundice revealed 107 cases in Conakry. Among them, 16 were diagnosed with acute leptospirosis. Because this disease is probably underdiagnosed in this country, a pilot study was undertaken in 2004 in Conakry, with an investigation of both humans and small mammals. Sera from 1200 human subjects were screened for leptospirosis antibodies to estimate the incidence of the infection during the past rainy season and identify risk factors for transmission. In parallel, rodents were trapped in households to identify the reservoir animals of the disease.
Methods: A cross-sectional serologic survey was carried out in 5 resource-poor urban neighbourhoods in Conakry, Guinea. A detailed standardised questionnaire was completed to document demographic and environmental risk factors of transmission. Leptospirosis specific IgM and IgG levels were detected by ELISA and confirmed with MAT testing. The trapped rodents were taxonomically identified and one kidney was collected for detection of leptospires by culture and PCR testing. A nested PCR with a high sensitivity was performed, non-pathogenic leptospires (e.g. *L. biflexa*) were excluded by primer design. PCR positive samples were confirmed using different typing PCRs.
Results: Approximately 7 percent of study subjects were positive for leptospira antibodies. Preliminary epidemiological analysis revealed as risk factors for leptospira IgM antibodies: (i) living in a neighbourhood from which leptospirosis cases were reported in 2001; (ii) use of tap water for washing and bathing; (iii) living close to a waste pipe; (iv) history of hospitalisation during the past rainy season
330 rodents were trapped within the 5 neighbourhoods. *Rattus rattus* and *Mus musculus* were the most frequent species, but in addition some *Crocidura* and *Mastomys* spp. were identified. In five of the kidney samples leptospiral DNA was detected by nested PCR. In three cases specific molecular typing revealed *L. kirschneri*.
Conclusion: This survey shows that a significant percentage of the population of Conakry, a city with ~2 Million inhabitants, is exposed to leptospirosis during the rainy season. Transmission probably occurs through leptospira infested water on the domestic compounds, with rodents as one possible reservoir. As the outbreak in 2001 shows, there is an urgent need for further identification of the main reservoir(s) of the disease and environmental control measures.

O252 Majority of the clinical *Yersinia enterocolitica* isolates in Finland belong to biotype 1A

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Objectives: Zoonotic *Yersinia enterocolitica* is a more common cause of gastroenteritis in Finland than domestic salmonellae. Annually, more than 500 *Y. enterocolitica* infections are notified in the register for infectious diseases. Most of the cases seem to be sporadic but occasionally outbreaks emerge, pigs being a common reservoir. *Y. enterocolitica* diagnostics is challenging since among the strains pathogenity varies. In addition, strains of other *Yersinia* species can resemble pathogenic *Y. enterocolitica* strains in their biotypic and serotypic reactions. Our purpose was to collect an extensive number of clinical strains, study their detailed phenotypic and genotypic characteristics, and interview the patients to assess the clinical significance of the different *Yersinia* strains.
Methods: We collected all *Yersinia* strains from10 Finnish clinical microbiological laboratories in the year 2006. The strains were examined by biotyping, serotyping, and by several genotypic methods, such as gene sequencing and pulsed-field gel electrophoresis. The patients were asked e.g. about onset and symptoms of illness, and food consumed. We then combined the data collected from bacterial strains with the information gathered from patients. To study the appearance of *Y. enterocolitica* in healthy population, we also studied stool samples of 200 healthy individuals.
Results: Approximately 40 *Yersinia* strains per month were received from the clinical laboratories. The majority of all strains, approximately 70%, belonged to *Y. enterocolitica* biotype (BT) 1A and 15% of the strains were of bio-/serotype 4/O:3 or 2–3/O:9. The remaining 15% consisted of other *Yersinia* species. The use of cold-enrichment increased the number of BT 1A findings. The symptoms of the patients with either a BT 1A or 4/O:3 finding were rather similar concerning abdominal pain and diarrhoea, but there was a statistically significant difference in appearance of fever in the patients with the 4/O:3 finding. Less than a one percent of the healthy individuals had *Y. enterocolitica* in their stool samples.
Conclusion: Majority of the Finnish clinical *Y. enterocolitica* findings were BT 1A strains that have traditionally been considered as non-pathogenic strains since they do not possess pYV virulence plasmid. However, according to the preliminary analyses of the patients' interviews some of the BT 1A strains were associated with severe gastrointestinal symptoms.

O253 Epidemiology of *Haemophilus influenzae* serotype A from 2000–2005, an emerging pathogen in Northern Canada and Alaska

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Background: Prior to introduction of the *Haemophilus influenzae* type b (Hib) conjugate vaccines, rates of Hib disease among aboriginal people living in Alaska (AK) and Northern Canada (N Can) were among the highest reported in the world. Routine vaccination has reduced these rates to very low levels; however, serotype replacement with non-type b strains may result in a reemergence of invasive disease in children.
Methods: We reviewed population-based data on invasive Hi disease in AK and N Can collected from 2000–2005 through the International Circumpolar Surveillance (ICS) network. Chart reviews were conducted on laboratory-confirmed cases using standardised forms to verify illness episode information. All Hia isolates were characterised using pulsed-field gel electrophoresis (PFGE). AK and N Can estimated populations as of 2005 were 655,435 and 132,956 respectively; aboriginal peoples comprised 19% of the population in AK and 59% in N Can.
Results: During the study period, a total of 138 cases of invasive Hi disease were reported from AK (76) and N Can (62). Among the 88 (67%) invasive Hi cases with serotype information available, 42 (48%) were serotype a, 27 (31%) were serotype b, 12 (14%) were serotype f. Among Hia isolates, 35 (83%) occurred in aboriginal peoples; median age was 1.1 years (range 3 mo to 74 years); 62% were male. Two Hia cases (1 adult/1 child) were fatal. Common clinical presentations included: meningitis (33%), pneumonia (29%), and septic arthritis (12%). There were no cases of epiglotittis. Overall annual Hia incidence was 0.9 cases per 100,000 population. Annual incidence rates in aboriginals in AK and N Can were 1.1 and 4.6 per 100,000 persons, respectively; rates in aboriginal children <2 years of age were 22 and 101 cases per 100,000 persons, respectively. PFGE analysis revealed genetically similar Hia strains in both AK and N Can.
Conclusions: Serotype a is now the most common Hi serotype seen in the North American Arctic, with the highest rates among indigenous children. Further research is needed to determine sequelae, risk factors, outbreak potential, and the utility of chemoprophylaxis for this disease.

O254 Mediterranean spotted fever: a reemerging rickettsiosis? New trends in epidemiology, ecology and clinical presentation

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Objectives: In recent years, rickettsial disease had undergone important evolution, particularly in the field of molecular genetics. In parallel, important changes in our comprehension of Mediterranean spotted fever (MSF) had occurred in the field of ecology, epidemiology, occurrence of severe forms. The objective of this study is to determine new trends in Mediterranean spotted fever.
Methods: We disposed of the data from the 'Unité des Rickettsies' from 1982 until today and of the data from 1993 to 2005 in Oran. We reviewed the literature from the first description of MSF to September 2006 on the subject to determine incidence of the disease, incidence of severe cases, risk factors of severity, changes in clinical description. We also reviewed literature on the state of knowledge about factors influencing the incidence of MSF, the vector and reservoir of *R. conorii*.
Results: First clinical descriptions based only on serology surely included infections related to multiple species of Rickettsiae and do not correspond to only one clinico-etiologic entity. Now, with more accurate technique of identification of rickettsial disease, we can differentiate different clinical presentation according to the species. Incidence of Mediterranean fever has known important variations with a peak in the 1980's. Incidence of severe form is also fluctuating; in Beja district, Portugal, the case fatality rate in hospitalised patients with MSF was 32.3%, the highest ever obtained there; in France, peak of incidence of MSF with 30 cases and of severe form (30%) was noted in 2004, one year after the canicule. The possible factors include an increased number of ticks, increased human contact with the habitat of infected ticks and climatic factors, such as the increase in temperature and the lack of rainfall. Multiple eschars are now recognized in MSF. In our unit, in 2004, 9 patients had a confirmed diagnosis of MSF either by PCR or culture of the eschar or blood culture. Among them, 3 had multiple eschars and 2/3 had a severe form of MSF. It is noteworthy that 6/9 of these patients had a severe form of MSF. In Oran, multiple eschars are not a risk factor for severe MSF.
Conclusion: MSF shows evolving features in epidemiology, clinical presentation and one hundred years after its first description, we better know the disease. However areas of uncertainty persist, such as what is the real vector and reservoir of MSF.

O255 Is *Echinococcus multilocularis* increasing in prevalence in the Western European border line?

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Objectives: Alveolar echinococcosis is one of the most pathogenic parasitic zoonoses in central Europe, caused after oral uptake of eggs of *Echinococcus multilocularis*, shed by infected foxes. At present, there seems to be a trend towards increased parasite density in central Europe and spread in western Europe, although it cannot be decided whether *E. multilocularis* recently has extended its habitat or whether the parasite has simply remained undetected until now in marginal regions. We analysed red fox data from an area close to the westernmost margin of *E. multilocularis* habitat in Europe, Belgium and a neighbouring province (Limburg) in The Netherlands (NL), with the aim of studying the emergence of the parasite in this area.
Methods: A total of 1202 foxes has been analysed (1018 in Belgium, 184 in NL) with 179 infected (164 in Belgium, 15 in NL) using mucosal scrapings. Spatial coordinates of the locations of infected and uninfected foxes have been determined by GPS. In addition, additional data in southern Europe were analysed using GIS and modeling to study changes in distrinution and prevalence.
Results: The large scale spatial distribution of the prevalence of infection among sampled foxes has been modeled as an ellipsoidal gradient, demonstrating increasing prevalence in southeastern direction that means towards the endemic area in central Europe. Using this gradient, the spatial pattern of *E. multilocularis* infection in Belgium and a contiguous region in NL could be shown to have a continuous distribution across national borders. Part of the Belgian data allowed investigation of the temporal changes in spatial distribution of *E. multilocularis*, revealing spreading into northwestern direction. A mathematical model describing the parasite population dynamics both in time and in space was fitted to the worm burdens of foxes sampled between 1996 and 2006 in NL. We found a strong indication that the parasite's reproduction number R_0 is greater than 1 and that the parasite is spreading to a wider region in Limburg. Based on the R_0 derived from the mathematical model of the parasite's transmission, we explore the effect of public health measures aimed to eradicate the infection.
Conclusion: Increased infection pressure of *E. multilocularis* in northwestern Europe is most likely to occur at present. In Belgium human alveolar echinococcosis had been absent in the past, but 3 human cases have been reported in 2004.

Novel resistance mechanisms and quinolone resistance

O256 Telavancin targets the bacterial membrane of *Staphylococcus aureus*: analysis of membrane effects by flow cytometry

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Objectives: Telavancin is a novel, multivalent, lipoglycopeptide antibiotic that exerts rapid bactericidal activity against a broad range of Gram-positive pathogens. Telavancin has a unique multifunctional mechanism of action that includes both inhibition of bacterial cell wall synthesis and disruption of bacterial membrane function. Previous studies with methicillin-resistant *Staphylococcus aureus* (MRSA) revealed that telavancin increases cell membrane permeability and causes rapid dissipation of the bacterial membrane potential. This study aimed to further characterise the effects of telavancin on the functional integrity of the MRSA cell membrane using flow cytometry.
Methods: Cell membrane potential and membrane permeability were studied in telavancin-treated *S. aureus* ATCC 33591 (MRSA) cells. The fluorescent dyes, DiOC2(3) and propidium iodide, were used to assess membrane potential and permeability, respectively. Flow cytometry was used to analyse uptake of these dyes in telavancin-treated cells.
Results: Telavancin (MIC, 0.5 μg/mL) caused a concentration- and time-dependent dissipation of the membrane potential in MRSA cells. After 60 minutes' exposure to telavancin, 67% of the bacterial population was depolarised at 8 μg/mL (approximate human plasma trough concentration), while 32 μg/mL resulted in 95% depolarisation. Membrane permeability was also concentration- and time-dependent, as seen when cells were exposed to telavancin concentrations ranging from 2 to 64 μg/mL, with 32 and 64 μg/mL yielding rapid, >90% permeabilisation of the cell population.
Conclusion: Exposure of MRSA cells to telavancin initiated concentration- and time-dependent membrane depolarisation and increases in permeability, further demonstrating that telavancin interferes with bacterial cell membrane function. These effects were observed at telavancin concentrations that are achieved clinically with 10 mg/kg intravenous dosing, and further highlight the therapeutic relevance of telavancin's multifunctional mechanism of action.

O257 Relationship between antibiotic exposure and overproduction of MexXY efflux pump in *Pseudomonas aeruginosa*

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Objectives: Overproduction of the MexXY efflux pump results in increased resistance to aminoglycosides (AGs), fluoroquinolones (FQs) and cefepime (FEP) in *Pseudomonas aeruginosa* (PA). This mechanism is very prevalent (up to 40%) in the clinical isolates. However, the conditions that favour the emergence of MexXY-overproducing mutants (PA/XY+) in the hospital setting remain unclear. We therefore analysed the temporal relationship between the prevalence of PA/XY+ and antibiotic use, and then compared the results to in vitro selection experiments.
Methods: The incidence of PA/XY+ per 1000 patient-days was determined between 2001 and 2004 (3,800 isolates) from the laboratory susceptibility database (% of isolates with a low-level resistance to all the tested AGs). We conducted time-series analysis of monthly incidence of PA/XY+ and monthly consumption of various antibiotic classes in our hospital. In parallel, the ability of antibiotics at 1 or 2 MICs to select for PA/XY+ mutants was determined in vitro with reference strain PAO1. Detection of PA/XY+ was done by replicating resistant clones on MH plates containing gentamicin (5 mg/L) or FEP (5 mg/L). The PA/XY+ in vitro mutants were further characterised phenotypically and genotypically.
Results: The adjusted Linear Transfer Function model showed a significant relationship between the AGs and the FQs use series and the PA/XY+ series. This was observed for contemporaneous data and with a lag of several months. Conversely a negative correlation was found between third generation cephalosporins (cefotaxime and ceftriaxone) or anti-pseudomonal penicillins use series and PA/XY+ series. There was no correlation between the FEP use, which is very low in our hospital (<0.5 DDD/100 PD), and PA/XY+ series. The model explained 70.1% of the variability of the resistance series (adjusted r-squared = 0.701). In vitro, PA/XY+ were readily selected by all the tested FQs (n = 4), AGs (n = 3) and FEP at a frequency of 3×10^{-7} to 5×10^{-6}.
Conclusion: This study strongly suggests that PA/XY+ are selected by AGs and FQs in the hospital setting. The broad-spectrum resistance of these mutants could reduce the efficacy of combination therapy involving substrates of the MexXY efflux pump (i.e. FEP, AGs and FQs).

O258 Telavancin inhibits peptidoglycan biosynthesis through preferential targeting of transglycosylation: evidence for a multivalent interaction between telavancin and lipid II

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Objectives: Telavancin (TLV) is a novel, rapidly bactericidal lipoglycopeptide with activity against methicillin-resistant *Staphylococcus aureus* (MRSA). TLV possesses a unique, multivalent, multifunctional

mechanism that contributes to enhanced activity and includes inhibition of cell wall peptidoglycan (PG) biosynthesis and disruption of bacterial membrane barrier function. In vitro studies suggest that TLV exhibits superior antibacterial potency relative to vancomycin (VAN); this may be due to the lipophilic decylaminoethyl side chain that targets TLV to the bacterial cell membrane. The objective of this study was to elucidate the mechanistic basis for the enhanced antibacterial action of TLV.
Methods: Isothermal titration calorimetry was used to determine affinities of TLV to 3-lipid II (a water soluble lipid II analogue), and wild-type lipid II embedded in model membrane vesicles made of 1,2-dioleoyl-sn-glycero-3-phosphocholine. PG biosynthesis was evaluated in methicillin-susceptible, methicillin-resistant, and vancomycin-intermediate isolates of *S. aureus*. Formation of immature (polymerised, non-cross-linked transglycosylation product) or mature (polymerised, cross-linked, the product of transglycosylation then transpeptidation) PG was assayed in intact cells by measuring incorporation of [^{14}C]-GlcNAc into 5% trichloroacetic acid-insoluble material in the presence of penicillin G (immature PG), or into hot 4% sodium dodecylsulphate-insoluble material (mature PG).
Results: The affinity of TLV for the water-soluble lipid II variant was $4.7\pm0.5\times10^{5}$ M^{-1}; a nearly 35-fold increase in affinity was observed with membrane-bound, wild-type lipid II. Inhibition patterns of immature and mature PG by TLV were distinct from those of VAN. VAN preferentially inhibited the formation of mature PG (transpeptidation reaction product), requiring ~10-fold higher concentration to inhibit transglycosylation. In contrast, TLV appeared to preferentially inhibit the transglycosylation reaction.
Conclusion: TLV inhibits PG polymerisation and cross-linking steps in bacterial cell wall synthesis. The higher affinity of TLV for membrane-bound lipid II (vs the soluble analogue) is consistent with the concept that TLV selectively binds to the bacterial cell membrane. The superior inhibitory potency of TLV toward PG biosynthesis may derive from its membrane-anchoring properties which confer increased binding affinity for the transglycosylase substrate, lipid II.

O259 Risk for selection by clindamycin of resistant mutants from *Staphylococcus aureus* inducibly resistant to erythromycin: erm(A) and erm(C) genes are not similar

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Objectives: *S. aureus* isolates inducibly resistant to erythromycin by production of ErmA or ErmC methylases are still susceptible to clindamycin. However, constitutive mutants resistant to clindamycin may be selected in vitro or in vivo in the presence of this antibiotic. The risk for selection may depend on several factors including the frequency of mutation to resistance, the bacterial inoculum size and the type of infection. Our objective was to compare the clindamycin mutation rate of clinical isolates of *S. aureus* containing an erm(C) or an erm(A) gene.
Methods: 28 isolates of *S. aureus* bearing erm(C) (n = 18) or erm(A) (n = 10), originating from various countries and including 12 community-acquired methicillin resistant strains representative of different clones, were studied. For determination of mutation frequencies, cells from an overnight broth culture were plated onto trypticase-soy agar plates supplemented with 20 mg/L of clindamycin. After 48 h of incubation at 37ºC, colonies were counted and the mutation frequencies were determined relative to the total count of viable organisms plated. Each experiment was repeated three times. Two erythromycin-susceptible *S. aureus* and 4 *S. aureus* with the efflux gene msr(A) were included as controls. Susceptibility to oleandomycin was determined by the disk diffusion method.
Results: *S. aureus* erm(C) displayed a 14-fold higher mean frequency of mutation to clindamycin [range: 1.7×10^{-6} to 4.4×10^{-8}; mean: 4.7×10^{-7} ($\pm5.5\times10^{-7}$)] than the *S. aureus* erm(A) strains [range: 7.3×10^{-10} to 1.8×10^{-8}; mean: 3.4×10^{-8} ($\pm2.4\times10^{-8}$)]. The frequencies of mutations for the erm(A) isolates were similar to those for the controls [range: 1.3×10^{-9} to 1×10^{-8}; mean: 1.1×10^{-8} ($\pm6.4\times10^{-9}$)].
All isolates with the erm(A) gene were susceptible to oleandomycin whereas no inhibition zone was observed for the isolates with the erm(C) gene.
Conclusion: *S. aureus* with the erm(C) gene are more readily susceptible to develop constitutive resistance to clindamycin than those with the erm(A) gene, regardless methicillin resistance. Susceptibility or inducible resistance to oleandomycin was associated with the presence of an inducible erm(A) or erm(C) gene, respectively and might constitute markers of these genes. The risk analysis for the clindamycin use in therapy of infections due to *S. aureus* inducibly resistant to erythromycin might include the nature of the resistance gene.

O260 Multidrug-resistant *Escherichia coli* mutants selected with diazepam or lomefloxacin overexpressing soxS, sdiA, acrAB, TolC and PBP3

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Objectives: Multiple resistance of *Escherichia coli* to distinct classes of antimicrobials is usually attributed to simultaneous increased expression of AcrAB-TolC efflux system and loss of porin F generated by transcriptional activators marA, soxS or rob. AcrAB-TolC is also up-regulated by SdiA, an *E. coli* protein that regulates cell division.
In this study, multidrug resistant mutants selected in vitro with diazepam (DZ) and lomefloxacin (LOM) from two *E. coli* susceptible clinical isolates were characterised.
Methods: Mutants were selected with DZ or LOM at 2, 3, 4 or 6 × the MIC. Tolerance of strains to 10% cyclohexane was measured in liquid medium. Inner and outer membrane proteins (IMP and OMP) were analysed by electrophoresis on polyacrylamide gels. Penicillin-Binding-Proteins (PBPs) were detected with Bocillin FL and Imperial Protein Stain. Active efflux was evidenced by 0.05 mM carbonyl cyanide m-chlorophenylhydrazone (CCCP). The expression of the acrA, acrB, marA, soxS, rob and sdiA genes was studied by reverse transcription of total RNA and PCR of cDNA (RT-PCR), using gapA gene as internal control of expression. PCR products were separated on polyacrylamide gels and detected using silver staining. Ag100 strain (induced or non-induced with 5 mM salicylate or 0.2 mM paraquat) was the control strain.
Results: Nine different clones were selected with DZ or LOM. Selection frequencies were around 10^{-9} to 10^{-8}. The nine mutants increased resistance to quinolones, chloramphenicol and β-lactams (2–8 fold ceftazidime, cefpirome and aztreonam MICs). The presence of CCCP increased the susceptibility of mutants to antimicrobial agents. The OMP TolC increased in mutants, coinciding with their increased cyclohexane tolerance. Mutants and parent strains showed a high expression of acrA and acrB genes. Nevertheless, only mutants showed overexpression of sdiA and soxS and OmpF decrease. Neither parent strains nor mutants showed marA or rob overexpression. Increased PBP3 expression was detected in only the mutants selected with 6 × MIC of DZ or LOM.
Conclusions:

i. DZ (a non-antimicrobial drug) and LOM selected multiple antibiotic resistant mutants that overexpressed the same transcriptional regulators, soxS and sdiA, resulting in TolC increased expression in mutants.
ii. The OmpF decrease, which was generated by soxS overexpression, and increased TolC and PBP3 expression contributed to 2–8-fold increment in ceftazidime, aztreonam and cefpirome MICs in mutants.

O261 Role of the pentapeptidic proteins from Gram-positive bacteria as a possible source of Qnr-like quinolone resistance determinants

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Objectives: To study the role of Qnr-like pentapeptidic proteins of several Gram-positive species in resistance to quinolones in vitro.
Methods: *Enterococcus faecalis* V583 homologue QnrA protein sequence was used to search pentapeptidic proteins with different levels

of aminoacidic identity in the NCBI database. A PCR-based strategy was used to clone and express the genes coding for Qnr-like pentapeptidic proteins of *E. faecalis* ATCC 29212, *E. faecalis* V583, *E. faecium* BM4147, *Listeria monocytogenes* 868 (clinical isolate), *Clostridium perfringens* 455 (clinical isolate) and *Bacillus cereus* ATCC 11778 in pPCR-Script vector (Stratagene) in *Escherichia coli* DH10B. According to CLSI guidelines, MICs of nalidixic acid, ofloxacin, norfloxacin, ciprofloxacin, levofloxacin and moxifloxacin were determined for clinical strains, reference strains and *Escherichia coli* DH10B harbouring recombinant plasmids containing genes coding for the pentapeptidic proteins.

Results: The aminoacidic identity of Qnr-like pentapeptidic proteins of the Gram-positive strains compared to those of plasmid-mediated QnrA1, QnrB1 or QnrS1 quinolone resistance proteins ranged from 20% to 27%. When compared to the homologue of QnrA in *E. faecalis* V583, the aminoacidic identity ranged from 30% to 35%. All the clones obtained in *E. coli* DH10B expressing a pentapentidic protein from the Gram positives strains conferred reduced susceptibility to quinolones. The recombinant plasmids expressing pentapentidic proteins in *E. coli* DH10B increased the MICs from 4 to 12 folds for nalidixic acid, from 6 to 24 folds for ofloxacin, from 2 to 47 folds for norfloxacin, from 12 to 32 folds for ciprofloxacin, from 4 to 21 folds for levofloxacin, and from 4 to 32 folds for moxifloxacin.

Conclusions: The pentapeptidic proteins analysed confer reduced susceptibility phenotype in *E. coli*. These data could provide further evidence about the possible role of pentapeptidic proteins of different Gram-positive species in their natural resistance to quinolones. These Gram positives species could constitute a reservoir of Qnr-like quinolone resistance proteins.

O262 The mode of action of daptomycin against *Staphylococcus aureus*

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Objectives: The Silverman model for the mode of action of daptomycin (DAP) is based on the leakage of potassium ions (K^+), membrane de-energisation and subsequent cell death without lysis. However, it is unknown whether loss of K^+ is sufficient to cause cell death. In this study we determined the kinetics of both cell death and lysis of *S. aureus* treated with DAP to establish any relationships between the two.

Methods: Time-kill experiments were performed at various concentrations of DAP to determine the kinetics of cell death and samples were taken and prepared for scanning and transmission electron microscopy (EM). Culture samples were taken at intervals and the extracellular and intracellular amounts of ATP determined. The extracellular and intracellular levels of β-galactosidase (β-gal) in a *S. aureus* strain expressing β-gal in its cytoplasm were also measured.

Results: The time-kill experiments show DAP to be rapidly bactericidal at 32 mg/L. DAP at 4 mg/L shows a bacteriostatic effect for the first 60 min and then becomes slowly bactericidal. There is evidence for dose-dependent leakage of ATP and β-gal from cells treated with DAP, but this leakage occurs at very different rates. After 30 min of treatment with DAP at 32 mg/L, 90% of the total ATP has been released but only a negligible amount of β-gal. The percentage of β-gal released increases steadily over a period of hours, reaching 40% after 6 h and 90% after 24 h. After 30 min of treatment with DAP at 4 mg/L, 50% of the total ATP has been released and this amount continues to increase steadily. The leakage of β-gal is less apparent and after 24 h the cells have released less than 30%. The EM images show some cellular disruption in the first hour after DAP treatment, with this damage becoming more apparent and widespread over the following hours. The images clearly show that the rate and extent of cellular lysis is dose-dependent.

Conclusion: Our findings support the Silverman model for the mode of action of DAP and the hypothesis that it can cause rapid cell death without whole cell lysis. We have, however, shown that cell lysis does occur over time, through both EM images and the leakage of large amounts of β-gal. Our findings, while upholding the Silverman model, suggest that it is too simple to conclude that cell death is due only to loss of K^+ and membrane de-energisation. The rapid loss of ATP would be an equally likely cause of cell death.

O263 The correlation between resistance phenotype and expression levels of efflux pumps of *Pseudomonas aeruginosa*

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Objective: To study the effects of efflux pump inhibitors (CCCP and PAβN) on carbapenems in *P. aeruginosa* (Pa); to investigate the correlation between the resistance phenotypes and expression levels of efflux pumps of Pa and discuss the mechanism of different phenotypes in resistant Pa.

Methods: MICs of imipenem (IMP) or meropenem (MEP) combined with efflux pump inhibitors against IMP resistant strains were determined by agar dilution method. For 32 strains with different resistant phenotypes to IMP and MEP, the mRNA expression levels of three efflux pump genes (mexA, mexD, mexF) were quantified by real time fluorescent quantitative PCR.

Results: The resistance rate of IMP and MEP descended gently after combined with efflux pump inhibitors. The strains with sustained MICs reached over 50%. MICs of only 8 strains descended to 1/4 original MIC values. The amount of mRNA of efflux pump genes in ATCC27853 was defined as 1. The amounts of mexA in susceptible strains were from 0.0003 to 0.99, those of mexD were from 0.0001 to 1.11, and those of mexF were from 0.013 to 0.31. The amounts of mexA in IMP resistant but MEP strains were from 0.28 to 4.48, mexD were from 0.37 to 20.06 and mexF were from 0.44 to 5.56. The amounts of mexA in both resistant strains were from 1.42 to 138.25, mexD were from 0.1 to 16.66 and mexF were from 0.22 to 12.61. That appeared overexpressions of efflux pumps accounted for 88.89% in 27 strains resistant to carbapenems. The statistic result showed the expression levels of three efflux pumps of strains resistant to carbapenems were much higher than susceptible strains.

Conclusions: When CCCP was incorporated in the Mueller-Hinton agar at a concentration of 5 μg/mL and PAβN was 20 μg/mL in vitro they could not obviously decrease the carbapenems resistance of Pa in this study, and there were rare relativity between the amount of mRNA of efflux pump genes and effects on MICs of carbapenems with efflux pump inhibitors. Overexpression of efflux pumps were the main reason for the resistance to carbapenems of Pa in our hospital. A phenomenon that manifold efflux pumps co-existed in a Pa isolate was common. It demonstrated that overexpression of MexAB-OprM plays an important role in resistance differences between IMP and MEP. Overexpressions of MexCD-OprJ and MexEF-OprN played important roles for carbapenems resistance in Pa, but had fewer effects on resistance difference between IMP and MEP.

O264 Natural variation within *Staphylococcus aureus* populations determines concentration and time-dependent phenotypic daptomycin resistance

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Daptomycin (DAP) is a rapid-killing lipopeptide antibiotic acting on MSSA, MRSA, and GISA strains. However, we have seen that, after killing, extended incubation with 8×MIC DAP concentrations results in a slow but sustained increase (from 24 h to 5 days) in viable staphylococcal cells. Such increase occurs in the presence of non-degraded DAP, and sub-cultivation of viable cells renders only DAP-susceptible organisms, suggesting heterogeneous phenotypic DAP-resistance.

Objective: To determine whether heterogeneous phenotypic DAP-resistance occurs as a result of natural variation in *S. aureus* populations before DAP exposure.

Methods: Time kill studies (in triplicate) with DAP 8×MIC were performed in 10 ml-tubes inoculated with 5×10^5 CFU/mL of 4 *S. aureus* ATCC strains (29213, 25923, 43300, and 700789) and incubated along 5 days. Natural variation in propensity to DAP-killing was studied with

the ATCC 25923 strain using the Luria-Delbrück fluctuation test (in duplicate) at 2×, 4×, and 8× DAP concentration (using both 3 ml- and 10 ml-tubes) along 6 days of incubation. Non-degraded DAP was determined by a microbioassay.
Results: In time kill studies, DAP displayed bactericidal activity against all ATCC strains at 3 hours. However, an after-killing increase (re-growth) of DAP-susceptible viable cells was observed along prolonged incubation despite of no DAP degradation over time. Fluctuation test indicates that natural phenotypic variation in propensity to DAP killing is generated at random before DAP exposure. The frequency of DAP-phenotypic resistant variants is inversely proportional to DAP concentration and time of exposure. Bacterial densities of 3×10^5 contain variants able to re-grow in 4 out of 6 tubes at 72 h after exposure to 2×MIC, only in 1/6 tubes at 4×MIC, and none (0/6 tubes) at 8×MIC. On the contrary, 1×10^6 bacterial density contains variants able to grow in all tubes at 2×, 4× and 8×MIC at 24 h. However, after 6 days of exposure, bacteria from 4 out of 6 tubes were killed.
Conclusions: A stochastic phenotypic variability in propensity to being killed by DAP occurs in *S. aureus*. The number of phenotypically resistant variants able to persist and re-grow in the presence of DAP is inversely proportional to drug concentration and time of exposure. Studies on putative implication of physiological, reversible heterogeneity in cytoplasmic membrane in this behaviour are awaited to interpret these findings.

O265 Worldwide dissemination of expanded-spectrum β-lactamase VEB-1 and quinolone resistance determinant QnrA1 through acquisition of IncA/C2 plasmids

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Purpose: To trace the plasmid co-dissemination of extended-spectrum β-lactamase gene blaVEB-1 and quinolone resistance gene qnrA by using the replicon typing method.
Material and Methods: Seventeen non-repetitive VEB-1 and/or QnrA-positive isolates (mostly transconjugants) have been included in the study. They had been collected from 1999 to 2005 from patients hospitalised in different parts of the world scattered on four continents. PCR-based replicon typing (PBRT) method including eighteen primer pairs was used, allowing to recognize FIA, FIB, FIC, HI1, HI2, I1-Ig, L/M, N, P, W, T, A/C, K, B/O, X, Y, and FII replicons. Plasmid restriction analysis followed by Southern hybridisation was performed to compare their structures.
Results: PBRT results showed that the thirteen blaVEB-1-positive plasmids (including eleven qnrA1-positive) were positive for the A/C-type replicon and sequencing identified the A/C2 replicon in all cases. By contrast, all the qnrA1-positive but blaVEB-1-negative isolates were negative for the A/C2 replicon. These results clearly indicated that the genes encoding QnrA1 and VEB-1, when identified concomitantly in a given isolate, were always located on plasmids belonging to the same IncA/C2-incompatibility group that may vary in size and digestion pattern. In addition, plasmids carrying the blaVEB-1 gene but lacking qnrA1 were also of the A/C2 type. On the opposite, plasmids that were qnrA1-positive but blaVEB-1-negative were of distinct replicon types, suggesting independent acquisition of the qnrA gene on different plasmids.
Restriction pattern analysis of plasmid DNAs performed using the PstI endonuclease revealed that the blaVEB-1-positive plasmids exhibited different restriction profiles but also sharing common bands, likely corresponding to a common plasmid backbone. Hybridisation performed with an A/C2-specific probe showed an identical signal revealing that the bands carrying the replication control region of the plasmids were of identical size, as expected with plasmids from the same lineage.
Conclusion: It is shown here that the IncA/C2 plasmid may be the main vehicle of the blaVEB-1 gene on which the QnrA1 determinant may be added.

Community-acquired bacterial infection I

O266 Risk factors for invasive group A streptococcal infections in Europe

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Objectives: Data on invasive group A streptococcal (iGAS) infections collected by 11 European countries were analysed to identify the frequency of factors predisposing individuals to infection.
Methods: Prospective surveillance of iGAS disease diagnosed between 1st January 2003 and 31st December 2004 was undertaken across Europe as part of the EU FP-5 funded Strep-EURO project (Cyprus, Czech Republic, Denmark, Finland, France, Germany, Greece, Italy, Romania, Sweden, UK). Each participant undertook enhanced surveillance of iGAS disease using a standardised case definition: isolation of GAS from a sterile site or from a non-sterile site in patients with clinical signs of streptococcal toxic-shock syndrome (STSS). Rates of iGAS disease were calculated for all countries with definable catchment populations (Cyprus, Czech Republic, Denmark, Finland, Romania, Sweden, UK).
Results: During 2003–04, 5538 iGAS cases were identified across the eleven countries. Cases were concentrated in the elderly and to a lesser extent in infants, with rates being higher in males than females in all countries except Denmark, where rates were non-significantly higher in females (RR = 1.16; 95% CI: 0.91–1.49). The age distribution of cases in the UK was unusual in having relatively high rates in young adults (3.35/100,000 in 20–39 year olds) compared to other countries, a reflection of the high proportion of UK cases being injecting drug users (21%). Seasonal patterns of iGAS infection were evident, with marked peaks in March/April in both years and an additional peak in January 2004. Considerable congruence in the timing of seasonal peaks and troughs was evident between countries. Eight per cent of infections were healthcare-associated, 63% of these post-surgical. Skin lesions/wounds were the most common predisposing factor, reported in 23% of cases. Four per cent of cases presented with puerperal sepsis, with the emm28 strain being strongly associated with these infections compared to other presentations (33% vs 10%; $Chi^2(1df) = 52.90$; $p < 0.001$). Eighteen per cent of cases were reported as not having any predisposing factors for iGAS infection.
Conclusion: Comparison of data from participating countries within the Strep-EURO programme is yielding interesting new perspectives on risk factors for iGAS disease which warrant further exploration. Understanding these patterns will help to identify potential targets for public health intervention.

O267 Incidence and management of group B streptococci colonised women in labour

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Objectives: Controversy still surrounds the indications for intrapartum antibiotic prophylaxis (IAP) against group B streptococci (GBS) for women in labour, with arguments for and against the two recognized care pathways; antenatal screening and risk factor assessment.
Using data obtained from a large test accuracy study into rapid detection of intrapartum GBS colonisation, the aims were to determine (1) the prevalence of vaginal and rectal GBS in labouring women, comparing standard culture to enrichment culture techniques; (2) what proportion of women present with different risk factors; (3) how both culture positivity and risk factors correlate with the frequency of IAP administration; (4) baby colonisation rates with regard to maternal colonisation site and whether or not IAP was given.
Methods: Vaginal and rectal swabs collected from 1185 women in labour for direct culture on streptococcal selective agar (CNA) and enrichment culture (LIM broth). 1125 ear swabs from neonates were collected within

1 hour of birth for enrichment culture. Data on risk factors and antibiotic therapy collected prospectively.

Results:

1. Enriched culture gave an overall (vaginal and/or rectal) colonisation rate of 21% compared to 10% with standard culture.
2. 32% of women studied had at least one risk factor, most commonly prolonged rupture of membranes (49%).
3. 50% of women with any risk factor did not receive IAP (Table 1).
4. Only 21% of GBS carriers received IAP, whilst 67% of women who received IAP were GBS-negative (Table 2).
5. The baby colonisation rate was 8% (90/1125). Positive vaginal culture identified 78% of the positive babies; positive rectal culture 89% and both rectal and vaginal positive culture combined 93%.

Table 1

Antibiotics risk factor	IAP	
	YES	NO
YES	136	135
NO	25	913

Table 2

Antibiotics GBS culture	IAP	
	YES	NO
POSITIVE	51	198
NEGATIVE	104	832

Conclusions:

1. The prevalence of GBS in labouring women was higher than in many previous European studies.
2. Our data confirms that the need for enrichment culture of vaginal and rectal swabs for maximum diagnostic yield.
3. There was a large percentage of women with risk factors who did not receive IAP, whilst only one third of women carrying GBS received IAP.
4. Rectal culture performs better than vaginal, although ideally they should be combined.

O268 An outbreak of pneumonia caused by *S. pneumoniae* at a military training facility in Finland in 2006

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Objectives: A cluster of pneumonia cases occurred among military recruits following a one-week hard encampment in Eastern Finland in the summer of 2006; several recruits were hospitalised and some had positive blood cultures for *S. pneumoniae*. To assess the extent of the outbreak and the carriage rate and microbiological characteristics of *S. pneumoniae*, all recruits who had participated in the encampment were screened for nasopharyngeal carriage of aerobic flora.

Methods: Nasopharyngeal cultures were taken from all 43 recruits who had participated in the military encampment and blood cultures were taken from all hospitalised recruits. All *S. pneumoniae* isolates were studied for antibiotic susceptibility, serotyped by latex agglutination and/or counterimmunoelectrophoresis, CIEP, and genotyped by multi locus sequence typing, MLST. Medical records of the hospitalised recruits were reviewed and all recruits were interviewed regarding the preceding symptoms, medical history, medication, and smoking habits.

Results: Of the 43 military recruits, five (12%) were hospitalised with pneumonia and two (5%) of them were positive for *S. pneumoniae* by blood culture. These two isolates were both of serotype 7F and ST2331. Eighteen (42%) of the 43 men were detected positive for *S. pneumoniae* by nasopharyngeal culture. All the 18 pneumococcal isolates were susceptible to penicillin and other commonly used antibiotics. Nine (50%) of the 18 isolates were of serotype 7F, five (28%) were of serotype 9N, three (17%) were of serotype 23F, and one (6%) was of serotype 16. MLST results corresponded with serotype results: all 7F serotypes were of ST2331, the 9N serotypes were of ST525, the 23F serotypes were of ST36, and the serotype 16 was of ST30. Three of the 18 pneumococcal carriers had no preceding symptoms while 15 presented with fever, rhinitis or sore throat. None had any underlying conditions known to be a risk factor for invasive pneumococcal disease.

Conclusions: Pneumococcal serotype 7F, ST2331, was clearly associated with the outbreak of pneumonia and nasopharyngeal carriage in this military encampment. Outbreaks of invasive pneumococcal disease can occur in a crowded environment such as military training facility even among previously healthy young men.

O269 Methicillin-resistant *Staphylococcus aureus* producing Panton-Valentine leukocidin: a cause of acute osteomyelitis in children

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Objectives: *Staphylococcus aureus* remains the prevalent bacterium causing acute osteomyelitis in children. The involvement of methicillin-resistant *S. aureus* (MRSA) as a cause of acute childhood osteomyelitis was investigated.

Methods: Included in the study were all children treated for acute osteomyelitis due to *S. aureus* in Western Greece from January 2005 until August 2006. Bone scan, MRI and X-rays were performed in order to ascertain the diagnosis and to assess the severity of the disease. *S. aureus* was isolated from blood and/or bone tissue when surgical drainage was necessary. The Staphylococcal Cassette Chromosome (SCCmec) type and Panton-Valentine leukocidin (PVL) genes (lukS-PV and lukF-PV) were detected by PCRs. MRSA clones were defined by PFGE of SmaI DNA digests. Signs, symptoms and laboratory findings were prospectively registered and statistically evaluated (Mann-Whitney-U test) in all patients until cessation of activity of the disease.

Results: Nineteen patients, 12 males and 7 females, median age 11 years, range 2–13 years, were diagnosed as having acute staphylococcal osteomyelitis. Three children had a suspected site of bacterial entrance (injury, burn, insect bite). *S. aureus* was isolated from the blood of nine patients, from the tissue of nine more patients and from both clinical specimens in one patient. In five children community-acquired MRSA (CA-MRSA) carrying SCCmec type IV and PVL was identified. Among the 14 MSSA isolated from the remaining patients, two were PVL-positive. The maximal ESR and CRP values as well as the time necessary for normalisation of ESR and CRP differed statistically significantly in patients with PVL positive stains (MRSA and MSSA) compared to PVL-negative MSSA ($p < 0.05$). Surgical drainage was more often necessary among patients with PVL-positive stains.

Conclusions: PVL-positive CA-MRSA are recovered not only from patients with superficial but also with invasive musculoskeletal infections. The production of PVL seems to be the main factor that contributes to the course of acute osteomyelitis.

O270 Methicillin-resistant *Staphylococcus aureus* clones producing toxic shock syndrome toxin 1: first case of intrafamily transmission

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Background: in the past decade, new strains of MRSA have emerged in the community, causing aggressive infections in young, otherwise healthy people. Most of community MRSA (C-MRSA) isolates expressed Panton-Valentine leukocidin (PVL), a highly potent toxin previously implicated in theses types of infections. Recently, a new clone of

C-MRSA producing toxic shock syndrome toxin 1 (tsst1) have been isolated [Durand G et al., J Clin Microbiol 2006]. Here, we describe the first report of family transmission of C-MRSA PVL-, tsst1+, in a patient (pt) with osteomyelitis.
Case report: In October 2006, a 29-year-old alcoholic man with no medical history was admitted in our department of infectious diseases for acromioclavicular osteoarthritis. He was married and had two children (6 and 8 year-old). In 2005 he had a traumatic dislocation of the right acromioclavicular joint and was operated one year later for tendons refection using homogenic graft. Two weeks after surgery, a purulent discharge from the post-operative wound was noted, and MRSA was cultured from swabs. The strain was resistant to oxacillin, kanamycin, tobramycin, and susceptible to ofloxacin, trimethoprim-sulfamethoxazole, pristinamycin, clindamycin, vancomycin, and was intermediate to fusidic acid. He was given oral pristinamycin (1 g tid for 8 weeks). One week after the end of treatment, in October 2006, he was admitted for a right acromioclavicular osteoarthritis which required surgical debridement and intravenous vancomycin treatment. A C-MRSA strain PVL– tsst1+ grew from intra-operative samples. The family carriage screening (including the pt) showed that the pt's children were colonised with the same MRSA strain according to the antibiotic susceptibility profile (PVL and tsst 1 detection still in process). The members of the pt's family had no healthcare associated risk factors for C-MRSA. Vancomycin was switched to oral trimethoprim-sulfamethoxazole and rifampicin combination for 3 months.
Discussion: In 2002, a new type of MRSA producing tsst1 has been described in France and Switzerland. To the best of our knowledge, family transmission of C-MRSA PVL-, tsst1+ strain has never been reported. In the present case report, a cross contamination between the pt and his family is highly suspected.
Conclusion: the present case shows that C-MRSA PVL-, tsst 1 might be associated with family transmission.

O271 *Legionella* spp. causing community-acquired pneumonia in Germany. Data from the Competence Network for Community-Acquired Pneumonia (CAPNETZ)

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Objectives: Prevalence, clinical data and outcome of *Legionella* pneumonia (LP) in 2503 patients from ten clinical centres with community acquired pneumonia (CAP), participating in the German multicentre study of the Competence Network for Community Acquired Pneumonia (CAPNETZ) were analysed.
Methods: All demographic, clinical and diagnostic data of the patients were recorded using standardised web-based data sheets. Work up of respiratory samples included identification of bacterial microorganisms as well as PCR for *C. pneumoniae*, *M. pneumoniae*, *Legionella* spp. and viral pathogens. Urine was tested for the presence of *S. pneumoniae* and *L. pneumophila* antigen. Relevant results from serologic tests were considered.
Results: *Legionella* spp. was diagnosed in 105 (4.2%) patients and thus represented after *S. pneumoniae* the second most frequent identified pathogen causing pneumonia. Patients with LP were predominantly older men, suffered more often from diabetes, were heavier smokers and produced more often respiratory samples, which were less often purulent. They received more often antibiotic substances with activity against atypical bacteria. Although 85% of the patients had a CRB 65 score of 0 or 1, respectively, 14 patients (13.3%) with LP expired.
Conclusion: *Legionella* spp. is a relevant pathogen causing CAP in Germany and is associated with a serious clinical course.

O272 Pathogenic *Yersinia enterocolitica* widely distributed in finishing pigs at slaughter in Switzerland

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Objectives: Yersiniosis is a common human infection, which is a reportable disease since 2001 in Germany. *Y. enterocolitica* 4/O:3 is the third most frequently isolated pathogen from patients with enteric disease after *Campylobacter* and *Salmonella*. The isolation rates of *Yersinia enterocolitica* of bioserotype 4/O:3 in German pig tonsils has shown to be high (56–67%). Only very few data are available for Switzerland. This work was conducted to study the prevalence of pathogenic *Y. enterocolitica* in Swiss pig tonsils. Furthermore, the isolates were characterised by pheno- and genotypic methods to get more epidemiological information to the current situation
Methods: Tonsils of 212 finishing pigs were sampled from a slaughterhouse in Switzerland during February and March 2006. Seventeen to 35 samples were collected from at least two different herds at 8 different days. The culture method included direct plating on a selective CIN plate, overnight enrichment in non-selective CASO bouillon and selective enrichment in ITC bouillon. The *Y. enterocolitica* isolates were bio- and serotyped. The pathogenicity was confirmed with pheno-and genotypic methods. ail-Positive *Y. enterocolitica* was detected with real-time PCR after overnight enrichment in CASO. At least one isolate per sample was characterised with PFGE using NotI enzyme.
Results: The prevalence of ail-positive *Y. enterocolitica* was 84% with PCR. The prevalence varied from 61% to 100% between the sampling days. The isolation rate was only 34% and varied between 18% and 82%. Bioserotype 4/O:3 was found in 96% of the culture-positive tonsils. Only 10% of the samples were biotype 2/O:9 positive. Six genotypes were obtained among bioserotype 4/O:3 isolates and 3 among bioserotype 2/O:9 isolates. Two genotypes (GT 1 and 2) of bioserotype 4/O:3 were dominant. Genotypes 1 and 2 were found on 6 and 5 out of 8 sampling days, respectively.
Conclusions: The prevalence of pathogenic *Y. enterocolitica* in Swiss pigs was high using PCR, however, the isolation rate was clearly lower than in German pigs. Most of the Swiss pigs were infected with bioserotype 4/O:3, which is the most common type found among human Y. enterolitica gastroenteritis in German and Switzerland and is the only type isolated from German slaughter pigs. Pig was also shown to be a reservoir for bioserotype 2/O:9 in Switzerland. The genetic diversity of ail-positive *Y. enterocolitica* in the pig tonsils collected from one Swiss slaughterhouse was limited.

O273 Risk factors, including antibiotic use, at hospital level for outbreaks with *Clostridium difficile* PCR ribotype 027

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Objectives: After reports on outbreaks of severe *Clostridium difficile*-associated disease (CDAD) in Canada, the USA and the UK, epidemics of this strain (PCR ribotype 027, toxinotype III) were first reported in The Netherlands in June 2005. National surveillance and typing of strains was started. Fluoroquinolone use has been recognized in several studies as an important risk factor at patient level for CDAD due to type 027. This study aimed at identifying risk factors at hospital level for outbreaks with type 027.
Methods: Data from 7 hospitals with known transmission of type 027 (group A), 5 hospitals with sporadic cases (group B) and 12 hospitals from a random selection without known type 027 (group C) were included. Quarterly data for 2004–2005 were collected on CDAD-incidence, inpatient antibiotic use (penicillins with extended spectrum and with β-lactamase inhibitors, cephalosporins, carbapenems, macrolides, clindamycin and fluoroquinolones) and hygienic and other preventive measures. The first 6 quarters were deemed the 'pre-epidemic' phase. In the last 2 quarters of 2005, the 'epidemic' phase, almost all

027 outbreaks took place. The association of AB use and hygiene policy with the incidence was analysed with multilevel linear regression.
Results: Mean pre-epidemic incidence in affected hospitals was 3.6 per 10,000 patient days, in group B 3.2 and in group C 2.3. During the epidemic phase this was 5.4, 3.3 and 2.8, respectively.
Pre-epidemically the unaffected hospitals had a significantly higher total use of the investigated AB [3776 DDD/10,000 patient days (pd)] than the A and B hospitals (3291 and 3325). In the epidemic phase, group A hospitals had significantly reduced their use of fluoroquinolones (from 615 to 416) whereas this rose in group C from 791 to 994 DDD/10,000 pd.
In multivariate analysis giving special instructions to visitors of diarrhoea patients (regression coefficient [r.c.] –1.9), wearing an apron when caring for diarrhoea patients (r.c. –2.7) and being an academic hospital (r.c. 1.6) were significantly associated with the CDAD incidence.
Conclusion: Hospital-wide use of most antibiotics known to be a risk factor for CDAD at patient level, such as fluoroquinolones, are not associated at institution level with higher incidences of type 027-associated CDAD. Possibly, investigation at ward-level might correlate better. Wearing an apron and giving special instructions to visitors in case of diarrhoea appeared to be protective.

O274 Amoebae as a second host for *Vibrio cholerae*

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Objectives: Acanthamoebae have been reported to represent the environmental host of several human pathogens. *Vibrio cholerae* requires 10^8 to 10^9 cells to cause cholera, and accordingly it needs an environmental host to enhance its growth. Our studies have shown that extracellular *V. cholerae* O1 and O139 have grown and survived in *Acanthamoeba castellanii*. The aim of this study is to examine the ability of *A. castellanii* to protect *V. cholerae* and to enhance its growth to be able to infect humans.
Methods: *V. cholerae* producing green fluorescent protein was co-cultivated with *A. castellanii*. An antibiotic assay used to examine ability of amoeba to protect intracellular bacteria and to differentiate between extracellular and intracellular bacteria.
A. castellanii cultivated with *V. cholerae* for one week. After gentamicin killing and washing of extracellular *V. cholerae*, the number of intracellular bacteria was found to be 2×10^5 cells/mL. The amoebae harbouring *V. cholerae* were re-cultivated and growth of the microorganisms examined by viable counts during 2 weeks.
Results: Gentamicin killed only the extracellular bacteria, while ciprofloxacin killed both, thus, the amoeba protected the intracellular *V. cholerae* from gentamicin. The antibiotic assay differentiated between extracellular bacteria that killed by gentamicin and intracellular bacteria that killed only by ciprofloxacin, which could diffuse into amoeba cells. Re-cultivation of *A. castellanii* harbouring intracellular *V. cholerae* resulted in enhanced growth of *V. cholerae* to 2×10^{11} CFU/mL and the bacteria survived for more than two weeks. Thus, amoeba acted as a biological incubator in which intracellular growth of the bacteria occurred to increase their number a million fold and maintained their overall viability. Existence of *V. cholerae* extracellularly as well as intracellularly in culture medium confirmed its facultative intracellular behaviour, which enhanced its growth and survival.
Conclusions: The utilised antibiotic assay differentiates between extracellular and intracellular bacteria. Free-living amoeba is a possible biological factor, which protects and enhances growth of *V. cholerae* to exceed its infections dose and to supports the idea of amoeba as a second host to the bacterium in nature besides man.

O275 Microbiology of thrombotic microangiopathies in Scotland, 2003–2006

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Without current surveillance of thrombotic microangiopathies such as HUS, neither prevalence nor outcomes are established in either adults or children. This study seeks to identify both health outcomes and existing management strategies. It comprises clinically driven enhanced surveillance of HUS and TTP and also further investigates the links between these syndromes and factors, which have been implicated in the etiology of HUS and TTP including infections, vascular procedures, chemotherapeutic agents and immuno-suppressants.
Cases were ascertained prospectively by active, national surveillance during 2003 to 2006. Consultants in haematology, infectious diseases, microbiology, nephrology, paediatrics and public health medicine, were sent a monthly e-mail with case definition and asked to indicate whether they had a 'case to report' or 'nil return'. Questionnaires, information sheets and consent forms were then sent to the relevant clinicians/consultants by post. All completed forms and questionnaires were returned to HPS and entered into a database for statistical analyses. From 2003 to October 2006, 145 reports of thrombotic microangiopathy were notified to HPS of which 103 were clinically designated HUS and 42 as TTP. There were 10 fatalities, 22 cases had some form of renal impairment and of those, 14 became dialysis dependent. Of 103 reports of HUS, 85 (83%) were caused by verotoxin-producing *E. coli* (VTEC) (82 were due to serotype O157). The non-O157 organisms were designated as serotypes O145, O177 and O-unidentifiable. Two atypical HUS cases were reported. One was preceded by parvovirus B19 infection/MMR immunisation and the other was due to infection with *Streptococcus pneumoniae*. The list of predisposing infections for development of TTP was more varied and included infection with coagulase-negative *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Clostridium difficile* but development of TTP was significantly associated with severe sepsis. The study clearly demonstrates that development of HUS or TTP has serious and sometimes fatal consequences and that infection is a major predisposing factor in development of such syndromes.

The medico-dental health interface – Paradigm shifts and advances in oral health for populations (Symposium organised by Colgate-Palmolive)

S279 Relationship between periodontal infections and systemic disease – the oral systemic connection

G.J. Seymour (Dunedin, NZ)

Common oral conditions, such as gingivitis and chronic periodontitis, are found world-wide and are among the most widely prevalent microbial diseases of mankind. A primary trigger for these conditions is the complex microbiota found as dental plaque, a complex microbial biofilm. Despite 3000 years of history demonstrating the influence of oral status on general health, recent decades have seen an accelerated effort for the prevention and management of these conditions through ground-breaking advances. One outcome of these advances is the realisation that periodontal diseases are associated with systemic conditions such as coronary heart disease and stroke; higher risk for preterm, low birth weight babies and pose threats to those with chronic disease: diabetes, respiratory diseases and osteoporosis. This presentation will highlight recent research associating periodontal disease with systemic diseases. The relationship between oral infections and systemic disease represents a paradigm shift in current research.
A portion of this lecture will be devoted to examining the role of chronic infections and their association with cardiovascular diseases (CVD). These infections include *Helicobacter pylori*, *Chlamydophila pneumoniae*, *Cytomegalovirus* (CMV), and, more recently, periodontopathic bacteria including *Porphyromonas gingivalis*. Although a number of potential mechanisms have been postulated, the mechanism by which these infections are associated with CVD is still unclear. The various hypotheses concerning this relationship include common susceptibility, inflammation via increased circulating cytokines and inflammatory mediators, direct infection of the blood vessels and finally the possibility

of cross-reactivity or molecular mimicry between bacterial and self antigens. The evidence for each of these mechanisms will be presented and the clinical implications for medical and dental practitioners discussed. This introductory lecture will set the stage for subsequent speakers highlighting the medico-dental interface as a required shift in health practices for the future.

S280 The importance of dentistry and medicine collaboration for the improvement of oral and general health

C. Migliorati (Fort Lauderdale, US)

While both medicine and dentistry are healthcare professions devoted to patient care, the interaction between the two disciplines remains limited. It is rare for dental professionals to be integral members of medical teams or hospital/healthcare settings. While referrals between the two professions occur, improved communications between professionals are required to optimise treatment options. This is increasingly required to capitalise on recent advances in health sciences for the management of chronic diseases that have reduced morbidity. Improvements in longevity have resulted in unique populations with special oral care needs and include those with cancer of the head and neck, immunocompromised, HIV/AIDS, geriatrics, residents of long-term care facilities, patients with lifelong conditions, after organ transplantation and those on prescription medications like those for blood-pressure control or anticoagulation therapy. This session will highlight the unique oral care needs for our changing patient population, and how oral health can be improved when dentists and physicians work together.
The role of prescription medications to treat medical conditions and their adverse reactions in the oral cavity will form a separate another area for discussion. Many medications, for instance, are xerostomic. Patients with xerostomia are at increased risk for tooth decay and periodontal disease. The need for increased collaboration between dentists and physicians to prevent or reduce complications by maintaining better oral and medical health will form a core theme for this session. It cannot be any longer ignored that oral health and medical health professionals must unite for optimal management of patient health.

S281 Common therapeutic approaches for the control of oral biofilms: microbiological safety and efficacy

P. Gilbert (Manchester, UK)

A primary step to improve oral health is the routine control of dental plaque, a natural biofilm. Oral hygiene formulations with significant effects on the dental biofilm augment ineffective practices of routine prophylaxis. Chlorhexidine and triclosan formulated in oral hygiene formulations have an extensive history of clinically proven efficacy and safety. An overview of clinically effective oral hygiene formulations and their role as therapeutic strategies will commence this presentation. With triclosan serving as a case-study, the talk will bring together recent advances in the assessment of oral biofilms, analyses of clinical strains including susceptibility to demonstrate the microbiological safety and efficacy of oral hygiene formulations.
The past three decades have witnessed no general reduction in the effectiveness of triclosan for its target bacteria including periodontal bacteria. Whilst triclosan is not noted for its activity against enteric bacteria and pseudomonads, laboratory studies demonstrated that chronic sub-lethal exposure of *Escherichia coli* can select mutant clones with significantly reduced susceptibility. These mutants are either mutated in an enzyme (FabI) associated with fatty-acid biosynthesis or over-express multi-drug efflux pumps. Initial concern that mutations in FabI might be capable of horizontal transfer between environmental bacteria and noscomial pathogens or that parallel processes might occur directly in Gram-positive pathogens have subsided. Similarly, concern about possible selection of resistance towards third-party agents (antibiotics) that might share the FabI gene target has proven unfounded. Selection of efflux-on mutants, whilst demonstrated in the laboratory is not supported by analyses of isolates from the hospital or domestic environment where agents such as triclosan have been widely deployed. Evidence suggests that efflux-on mutants are unable to compete in natural microbial communities.
Whilst there is insufficient evidence to suggest that the uncontrolled use of triclosan in domestic products is totally free of risk, its deployment for oral hygiene, especially where such use might limit the number of refractory infections and their possible complications contributes ultimately to a reduction in antibiotic use is encouraged.
Collectively, there is no evidence that the long-term application of triclosan formulations to the oral cavity or skin selects for triclosan-resistant bacterial populations.

S282 Clinical efficacy of triclosan/copolymer and other common therapeutic approaches

R. Davies (Manchester, UK)

As the increasing elderly population retain more of their natural teeth into later life periodontal disease will pose an increasing problem both for the dentition and potentially general health. The maintenance of an effective level of oral hygiene is the cornerstone of all attempts to prevent and control periodontal diseases and yet the widespread prevalence of these diseases indicates the inability of most people to achieve a level of plaque control commensurate with periodontal health. The inclusion of antibacterial agents, such as chlorhexidine and triclosan/copolymer, into oral care products has provided the consumer with the means to improve their oral health. Randomised controlled clinical trials have demonstrated that a dentifrice containing triclosan/copolymer significantly improves gingival health, prevents the onset of periodontitis and furthermore reduces the progression of the disease. These benefits were initially attributed to the antibacterial action of triclosan but evidence now suggests that it is also an anti-inflammatory agent. The delivery of such benefits is considerable with implications for the maintenance of oral and general health and the costs of dental care.

Current trends in treating continuously evolving Gram-negative infections (Symposium organised by Janssen Cilag)

S286 Treatments for serious Gram-negative infections

J. Rello (Tarragona, ES)

The emergence of multidrug resistance (MDR) in pathogens such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and Enterobacteriaceae that produce extended-spectrum beta-lactamases (ESBLs) is alarming. Increasing MDR in patients treated for nosocomial infections is causing an increase in costs, morbidity, and mortality. Traditionally, a narrow-spectrum drug was used first, and the most potent drugs were reserved for subsequent use. Unfortunately, the traditional approach increases the chance of treatment failure and the emergence of multidrug-resistant strains. In fact, the inappropriate choice of antibiotics is the most important independent risk factor for death.
ESBL producers are resistant to most penicillins and cephalosporins and are often cross-resistant to aminoglycosides and fluoroquinolones as well. Carbapenems have been shown to provide superior outcomes compared with other agents for infections caused by ESBL producers. Consequently, the first-line use of carbapenems is optimal for serious infections in which ESBL pathogens are suspected.
The major non-fermentative Gram-negative pathogen of concern is *P. aeruginosa*. *P. aeruginosa* is a common cause of nosocomial infections and the leading cause of ventilator-associated pneumonia. Initial therapy should include an antipseudomonal β-lactam, such as piperacillin-tazobactam, an antipseudomonal carbapenem, or an antipseudomonal cephalosporin, plus an aminoglycoside or a fluoroquinolone. *A. baumannii* generally is susceptible to carbapenems, aminoglycosides, sulbactam, colistin, or tigecycline. Carbapenems are one of the few classes that remain an excellent choice for both nonfermenters. Doripenem is a

new broad-spectrum carbapenem with activity superior to that of other carbapenems against *P. aeruginosa* and similar to other carbapenems against *A. baumannii*.

As antibiotic resistance increases, empiric therapy for severe infections should be based on local pathogen etiology and resistance patterns. A new approach is to start with a high-dose, broad-spectrum antibiotic and then tailor the individual therapy based on microbiological results. Carbapenems are likely to remain an important option for serious Gram-negative infections for the foreseeable future. Since the number of new intravenous agents with activity against Gram-negative organisms is extremely limited, the development of a new carbapenem may help to reduce the mortality, morbidity, and cost of serious nosocomial infections.

S287 Should all carbapenems be viewed the same?

D. Livermore (London, UK)

The widely marketed carbapenems long comprised imipenem (launched 1985) and meropenem (1996), but the family is now growing, with ertapenem (2002), doripenem (anticipated 2007/2008), and CS-023 (in development). Use is also growing, driven by the spread of extended-spectrum β-lactamases (ESBLs).

The carbapenems have much in common with each other, with broader activity and better stability to ESBLs and AmpC enzymes than other β-lactams, but with lability to metallo- and KPC β-lactamases and to the OXA carbapenemases of *Acinetobacter* clones. Three carbapenem groups nevertheless can be defined.

Group 1 agents lack activity versus non-fermenters; they are represented by ertapenem and perhaps in the future also by oral analogues. Ertapenem's utility lies in the treatment of community-acquired infections, where ESBL producers are likely or proven; it has convenient, once-daily, dosing but is the most vulnerable carbapenem to combinations of ESBL or AmpC plus impermeability.

Group 2 comprises imipenem, meropenem, and doripenem, plus analogues available only in East Asia; they are active versus most non-fermenters and are appropriate in nosocomial settings where these pathogens are likely. Doripenem and meropenem are very similar, but with doripenem circa two-fold more active versus most species; imipenem is slightly less active against Gram-negative bacteria except *Acinetobacter* but is the sole carbapenem active versus *Enterococcus faecalis*. Doripenem and meropenem need two mutations – loss of OprD and up-regulated efflux – for resistance in *Pseudomonas aeruginosa*. Imipenem resistance arises by loss of OprD alone and can be selected in therapy; nevertheless, imipenem is unique in evading pseudomonal efflux. Meropenem and doripenem are relatively stable to renal dehydropeptidase, but imipenem needs protection with cilastatin; doripenem and meropenem are chemically stable, allowing prolonged infusion, whereas imipenem is not; doripenem and meropenem have little seizure potential (meropenem is licensed for meningitis), but CNS side-effects limit imipenem dosage.

Group 3, comprising analogues active versus MRSA, is represented by the developmental agent CS-023; its minimum inhibitory concentrations for MRSA are more widely distributed than those of anti-MRSA cephalosporins, but it has the wider spectrum typical of a carbapenem.

As carbapenem usage expands, it is critical to understand these differences, so that the most appropriate analogues can be selected.

S288 Optimising utility of the carbapenem class

A. MacGowan (Bristol, UK)

Increasing antibiotic resistance and a lack of new drug classes in development are driving the need to optimise use of currently available drugs. The goal of antimicrobial chemotherapy is to optimise the combined pharmacokinetic (PK) and pharmacodynamic (PD) profile of a drug so that the greatest percentage of patients achieves the PD target associated with a favourable outcome, while minimising the development of resistant organisms. Integration of population PK, a PD target, and microbiologic surveillance data by Monte Carlo simulations can generate an empirical dosing strategy that maximises the likelihood that an antibiotic regimen achieves the desired PD end point (exposure target). For β-lactams, the exposure target is the percentage of time that free drug levels are above the minimum inhibitory concentration ($T > MIC$). For carbapenems, the $T > MIC$ requirement is lower than for other β-lactams. By extending the infusion period to achieve the necessary $T > MIC$, a lower dose can achieve the same efficacy as a higher dose, while lowering cost and potential toxicity. For example, a recent publication showed that a 1-g 0.5-h infusion of meropenem has a 77.1% rate of target attainment against *Pseudomonas aeruginosa* isolated from hospitals in Hungary, whereas a 3-h infusion of 0.5 g meropenem would have an 83.8% rate of target attainment. In a retrospective study that compared 0.5 h piperacillin/tazobactam infusions (3.375 g, q6h) with 4-h infusions (3.375 g, q8h) in patients infected with *P. aeruginosa*, the 4-h infusions reduced 14-day mortality from 31.6% to 12.2% ($P = 0.02$) and median length of stay from 38 to 21 days in patients with APACHE II scores $\geqslant 17$ ($P = 0.02$).

Doripenem is more stable upon reconstitution than other carbapenems, potentially making it more convenient and easier to use as an extended infusion. Furthermore, the study design for the doripenem ventilator-associated pneumonia phase 3 trial includes an extended infusion regimen (0.5 g over 4 h).

In conclusion, the same dose of an antibiotic has the capacity to increase efficacy for more resistant infections if infused over an extended period. Extended infusion of a lower dose of antibiotic may produce efficacy equivalent to shorter, higher-dose infusions while reducing both toxicity and the emergence of resistance. Extended infusion of a carbapenem, such as doripenem, that has neither seizure potential nor high resistance selection is a particularly promising strategy for serious infections.

S289 Doripenem: a new, potent, broad-spectrum treatment for serious Gram-negative infections

K. Naber (Munich, DE)

Doripenem is an investigational carbapenem that has completed phase 3 multi-national trials for complicated urinary tract infections (cUTIs), complicated intra-abdominal infections (cIAIs), hospital-acquired pneumonia (HAP), and ventilator-associated pneumonia (VAP). Doripenem's advantages over other carbapenems include: enhanced activity against *Pseudomonas aeruginosa* (with a low propensity for resistance), the lowest potential for seizures in the carbapenem class, and the greatest stability after reconstitution.

Two randomised, double-blind phase 3 cIAI trials compared intravenous (IV) doripenem (500 mg q8h) with IV meropenem (500 mg q8h), with an option to switch to amoxicillin/clavulanate for both treatment arms after $\geqslant 9$ doses of IV study drug therapy. Results from the cIAI trials showed that doripenem (500 mg q8h) is well-tolerated and non-inferior to meropenem (1 g q8h). Doripenem was microbiologically effective against major causative organisms of cIAIs, including Enterobacteriaceae and *Bacteroides* spp. Adverse events (AEs) occurring in $\geqslant 3\%$ of subjects in either treatment arm were nausea and vomiting. No seizures were reported.

A randomised, double-blind phase 3 cUTI trial compared IV doripenem (500 mg q8h) with IV levofloxacin (250 mg q24h), with an option to switch to oral levofloxacin for both treatment arms. Results from this trial showed that doripenem is well-tolerated and non-inferior to levofloxacin. Doripenem was effective against major causative organisms of cUTIs, including *Escherichia coli*, *Proteus mirabilis* and *Klebsiella pneumoniae*. Doripenem had greater efficacy in patients who remained on intravenous therapy and in patients with levofloxacin-resistant *E. coli*. Fewer pathogens had a 4-fold increase in minimum inhibitory concentration during therapy with doripenem than with levofloxacin. AEs occurring in $\geqslant 3\%$ of subjects in either treatment arm were headache, diarrhoea, and phlebitis. No serious study drug-related AEs were reported. Urine from patients was obtained and evaluated to assess urine bactericidal titers. Serum and urine drug concentration data collected in Phase 1, 2, and 3 studies will also be discussed.

Doripenem also completed a phase 3 trial versus piperacillin/tazobactam for HAP (including early-onset VAP), and a phase 3 trial versus imipenem for VAP (early and late onset). Doripenem's superior stability allowed the VAP trial to utilise an extended infusion (4-h) regimen for doripenem that may maximise for these difficult infections.

Keynote lectures

K290 Immune reactions to *Mycobacterium tuberculosis* – implications for new vaccines

S. Kaufmann (Berlin, DE)

Tuberculosis is a major health threat with 9 million new cases and 2 million deaths annually. The available vaccine, BCG, protects newborns from miliary tuberculosis, but fails to prevent the most prevalent form of disease, pulmonary tuberculosis in adults. One third of the world population is infected with the etiologic agent, *Mycobacterium tuberculosis*. Hence: (i) *M. tuberculosis* can be controlled (though not eradicated) by the immune response induced by natural infection; (ii) BCG fails to induce a protective immune response at least in those individuals who are susceptible to tuberculosis.

Current vaccination strategies have to consider both pre-exposure and post-exposure vaccines. Acquired immunity against tuberculosis is a T cell-dependent phenomenon. The T cell system comprises distinct populations. CD4 T cells are undoubtedly of central importance for acquired resistance against tuberculosis. Antigens of *M. tuberculosis* also stimulate CD8 T cells, probably through crosspriming. In addition, unconventional T cells also seem to participate in immunity against tuberculosis. What can we learn for rational vaccine design? Novel vaccination strategies either focus on subunit vaccines or viable attenuated vaccines. Subunit vaccination strategies are based on the assumption that one or few antigens suffice for an efficient immune response. Hence, the identification of protective antigens represents an essential prerequisite for the success of this type of vaccines. Subunit vaccines come in three forms: Protein/adjuvant formulations, recombinant heterologous carriers or naked DNA constructs. Viable attenuated vaccines are based on the assumption that multiple antigens are required for efficacious protection. Two major strategies are being pursued: Knockout mutants of *M. tuberculosis* and improved recombinant (r)-BCG vaccines. Improved r-BCG should first be endowed with a higher immunogenicity and second may need a broader antigenic repertoire. Rational vaccination strategies performed in the laboratory of the author focus on improved BCG. A r-BCG strain which expresses listeriolysin induces better protection than wild-type BCG. It is tempting to speculate that a prime/boost scheme comprising prime with improved r-BCG and boost with the most efficacious subunit vaccine candidate will provide optimal protection. Identification of a biosignature that allows distinction between infection/protection and infection/disease in tuberculosis could speed up efficacy testing of vaccines in clinical trials. The Grand Challenge 6 of the Bill & Melinda Gates Foundation aims at identifying such biosignatures.

Taking advantage of our increasing knowledge about the immune response to *M. tuberculosis* will facilitate rational design of novel vaccines against one of the most frightening threats in the world, tuberculosis. The following years will witness the transformation of vaccine candidates from preclinical to clinical trials requiring intensive coordination in order to identify the most appropriate candidate(s).

Recommended reading:

- Kaufmann SHE. Nat. Rev. Immunol. 6: 699–704 (2006).
- Kaufmann SHE. Trends Immunol. 26: 660–667 (2005).
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- Baumann S, et al. Curr. Opin. Immunol. 18: 438–448 (2006).

K291 Clinical implications of PK/PD

J. Mouton (Nijmegen, NL)

One of the most important characteristics that separate antimicrobials from other drugs used in clinical medicine is that their target (the receptor of the drug) is part of the microbe, and not of men. The activity of the antimicrobial can therefore be studied both in vitro and in model systems, such as animal models, and a relationship between exposure and effect established. The exposure is primarily determined by the pharmacokinetic characteristics of the drug, while the effect is determined by the concentration–effect relationship of the antimicrobial and the micro-organism. However, in most clinical settings, it is the potency of the antimicrobial that is measured in terms of the MIC and not the concentration–effect relationship itself. Therefore, a relationship needs to be ascertained between the MIC, exposure of the drug and effect. Over the last decade, these pharmacokinetic/pharmacodynamic (PK/PD) relationships of antimicrobials have been established for most classes of drugs. The two major indices describing these relationships are the time the concentration of the antimicrobial remains above the MIC (T >MIC) as a fraction of the dosing interval and the Area under the Time Concentration curve over 24 hours divided by the MIC (AUC/MIC ratio). The Peak/MIC ratio correlates with effect for a number of drugs, but it in many, if not all, cases the effect can not be clearly distinguished from the AUC/MIC effect. Importantly, for virtually all antimicrobials, it is the exposure of the free non-protein bound fraction of the drug that best correlates with efficacy, and is of particular significance when drugs are compared with each other within a class and/or across species, including men.

From these PK/PD relationships, a number of issues have come forward. Importantly, it has been shown in a number of clinical trials – now exceeding 10 – including several classes of drugs, that the PK/PD relationships that do exist in animals are, as expected, similar to those in men. Moreover, it has been shown that the quantitative relationships are remarkably similar. In other words, exposures in infected animals result in the same effect as exposures in infected humans under similar conditions. A second, perhaps even more important topic is that these relationships – once well established – can be used to optimise therapy in patients by optimising exposure of the drug. In a relatively simple approach, these relationships are used in daily clinical practice in the use of clinical breakpoints. These MIC values are used to discriminate between high and low probabilities of successful outcome of treatment (S and R, respectively) based on the PK/PD relationships of the antimicrobial together with the clinical setting. For instance, lower exposures to obtain the same effect are needed in the immunocompetent host because of the contribution of the immune system. More sophisticated, these relationships are used to adjust dosing regimens once the MICs of the infectious micro-organisms are known, and thus provide an integrated approach to the individual patient. Finally, PK/PD relationships are used in the development phase of the drug in determining the optimal dose for the indication sought. Alternatively, once dosing regimens are established, they can be used to form an opinion on the possible indications for various clinical treatment modalities.

Dissemination and reversal of antibiotic resistance

S293 Dissemination of resistance unrelated to antimicrobial consumption

G.M. Rossolini (Siena, IT)

Dissemination of microbial drug resistance observed in the antibiotic era is clearly related to the selective pressure generated by the use of antibiotics in clinical, veterinary and agricultural practices. This notion is universally acknowledged and supported by the several studies that have correlated the emergence and dissemination of resistance with the use of new antibiotics, and by documentation of the absence of acquired resistance in clinical isolates from the pre-antibiotic era.

However, the presence of antibiotic-resistant bacteria has recently been reported also in humans and in wild animals living in remote areas where antibiotic exposure has been absent or minimal [1–3]. This unexpected finding raises a question on the mechanisms responsible for spreading and maintenance of antibiotic resistance in similar settings, and could

have important implications for the design of strategies addressed at controlling bacterial resistance based on antibiotic restriction policies.
Results of studies on antibiotic resistance in settings of minimal antibiotic exposure (including investigations recently carried out in very remote human communities living in the Bolivian Chaco and in the Alto Amazonas jungle of South America, and among wild reptiles (land iguanas) from a remote and protected island of the Galápagos archipelago with no documented sources of antibiotic exposure and minimum human contacts) will be critically reviewed in this presentation, and the mechanisms potentially involved in the dissemination of resistant strains and resistance genes unrelated to antimicrobial consumption will be discussed. Results from the characterisation of resistance determinants carried by bacterial isolates from these settings pointed to their likely origin from antibiotic-exposed areas rather than to a local and independent resistance selection process. However, the mechanisms responsible for this flow of resistance genes and for their maintenance and spread in absence of sustained antibiotic use remain elusive.

Reference(s)

[1] Gilliver M et al. Antibiotic resistance found in wild rodents. Nature 1999; 401: 233-4.
[2] Bartoloni A et al. High prevalence of acquired antimicrobial resistance unrelated to heavy antimicrobial consumption. J Infect Dis 2004; 189: 1291-94.
[3] Grenet K et al. Antibacterial resistance, Wayampis Ameridians, French Guyana. Emerg Infect Dis 2004; 10: 1150-3.

P. aeruginosa pathogenesis and immunotherapeutics

O295 Alveolar macrophage phagocytosis and respiratory burst activity is regulated by Lyn–PI3Kinase–Akt pathway

S. Kannan, A. Audet, H. Huang, M. Wu (Grand Forks, US)

Introduction: Alveolar macrophages (AM) form the first line of defence in the lung alveoli. *Pseudomonas aeruginosa* (PA) is a common hospital-acquired infectious agent that can cause life-threatening infections in susceptible individuals. We hypothesise AM to have important function in early stages of PA infection.
Rationale: Recently, we have shown that Lyn tyrosine kinase is critically involved in alveolar epithelial cell invasion with PA through lipid raft mechanism. Lyn is known to be involved in mast cell motility and generation of superoxide free radicals in neutrophils. Thus we propose a role for Lyn in AM function during PA infection.
Methods: Akt activity was detected by immunoblotting with phospho Akt (Ser 473) antibody and by in vitro kinase assay. Lyn, PI3Kinase and Akt protein interactions were analysed by co-immunoprecipitation and Lyn-GST pull down assays. Phagosome formation and localisation of signaling proteins were studied by immunostaining with fluorescent antibodies followed by confocal microscopy. Lyn-YFP and PH-Akt-GFP co-transfected MHS cells were used for studying spatio-temporal association of Lyn and Akt during PA phagocytosis by live cell confocal microscopy. Phagosomes from infected cells were isolated by sucrose density gradient centrifugation for biochemical characterisation.
Results: PA-infection induced Akt activity in MHS cells depends on Lyn function. Lyn, PI3Kinase and Akt were actively recruited to phagosome fractions in PA-infected cells. Both Lyn-YFP and PH-Akt-GFP were found to colocalise in the lamellipodium and phagocytic cup of PA-infected MHS cells from live cell imaging studies. GST pull down assay confirmed Lyn–Akt interaction. Respiratory burst activity depends on Lyn function.
Conclusions: Our data indicates that Lyn–PI3Kinase–Akt pathway is crucial for regulating phagocytosis and respiratory burst activity of AM.

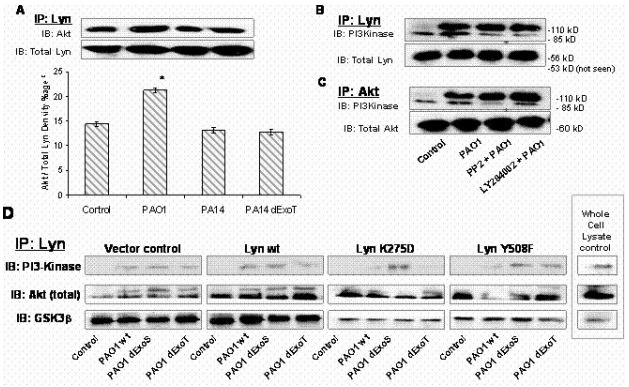

Figure 1. Co-immunoprecipitation analysis shows that Lyn interacts with PI3Kinase (B) and Akt (A) upon PAO1 infection of MHS cells. This interaction is significantly affected by pretreatment with PP2 (Lyn inhibition) or LY2094002 (PI3Kinase inhibitor) (B,C). (D) Lyn dominant negative transfection decreases Lyn and Akt association. GSK3β is a downstream effector of Akt.

O296 EF-Tu, a novel phosphorylcholine-containing protein involved in the interaction of chronic infectious *Pseudomonas aeruginosa* isolates with the airway epithelial cells

M. Barbier, L. García, A. Oliver, J.B. Goldberg, S. Albertí (Palma de Mallorca, ES; Charlottesville, US)

Pseudomonas aeruginosa PAO1 grown at 22ºC expresses a 43-kDa protein that contains a phosphorylcholine (ChoP) epitope. In other respiratory pathogens, like *Streptococcus pneumoniae* and *Haemophilus influenzae*, this motif interacts with the airway epithelial cells via Platelet Activating Factor Receptor (PAFr).
Objective: The objective of this study was to identify the 43-kDa ChoP containing protein and characterise its function in the virulence of *P. aeruginosa*.
Methods: To identify the ChoP containing protein, bacterial cell fractions were separated by FPLC. Fractions were analysed by Western blot using specific monoclonal anti-ChoP antibodies and those that contained the ChoP epitope were further purified by SDS-PAGE. A 43-kDa protein containing the ChoP epitope was cut out of the gel, trypsinised, and subjected to capillary LC-MS and MS/MS.
ChoP epitope expression of whole cell extracts of 92 genetic unrelated *P. aeruginosa* isolates (46 from chronic infections and 46 from acute infections) was analysed by Western blot analysis using specific monoclonal anti-ChoP antibody. ChoP epitope expression was also analysed by flow cytometry and immunofluorescent microscopy on intact cells of *P. aeruginosa*.
To determine whether the ChoP epitope was involved in the interaction of the *P. aeruginosa* isolates with the respiratory epithelial cells, standard invasion assays were performed using 16HBE14- bronchoepithelial cells, either treated or untreated with a PAFr antagonist.
Results: The 43-kDa ChoP containing protein was shown to be the elongation factor Tu (EF-Tu). The expression of the ChoP epitope at 37ºC was significantly more frequent in *P. aeruginosa* strains isolated from chronic infections (70%) than from acute infections (28%). The ChoP epitope was shown to be expressed on the outer surface of the bacterial cell and bacterial invasion was significantly inhibited by treatment with the PAFr antagonist compared to controls.
Conclusions: We have demonstrated that in *P. aeruginosa*, the ChoP epitope is associated with EF-Tu. A high percentage of the isolates from chronic infections, in comparison with those from acute infections, express this epitope at 37ºC. This epitope is present on the cell surface and mediates the invasion of the airway epithelial cells via PAFr.

O297 Development of recombinant antibody therapeutics for the treatment of bacterial infections

I.D. Broadbent, K.A. Charlton, G. Strachan, S.J. Williams, J. Steven, J. Park, C.J. Barelle, D.P. McGregor, A.J.R. Porter (Aberdeen, UK)

Objectives: We aim to develop novel therapies for the treatment of Gram-negative bacterial infections, by isolating monoclonal antibodies that are capable of binding to signal molecules involved in bacterial quorum sensing. Signalling blockade was hypothesised to prevent the production of virulence factors and so allow the host immune system to eliminate the infection.
Methods: As Gram-negative bacterial signaling molecules are typically of low molecular weight (less than 500 Da) they have been considered intractable to standard antibody discovery methodologies. Therefore we have used a novel suite of antibody discovery processes termed Haptomics® to isolate a panel of human antibodies capable of binding to signal molecules produced by Gram-negative bacteria.
Results: We have demonstrated the abilities of the antibodies to bind bacterial signaling molecules using a range of in vitro experiments, and we have also evaluated the antibodies using in vivo models of *Pseudomonas aeruginosa* infection. Results will be presented demonstrating the efficacy of our fully human recombinant antibody fragments and whole IgG molecules in the treatment of Gram-negative bacterial infections.
Conclusion: Our approach offers the exciting prospect of using powerful immunological reagents to combat bacterial infection. By targeting the signal molecules, rather than the bacteria themselves, we believe that resistance is considerably less likely to develop to these antibody-based therapeutics. We are currently broadening our approach to develop antibodies capable of disrupting quorum sensing in Gram-positive organisms.

O298 *Pseudomonas aeruginosa* utilises its type III secretion system to kill the free-living amoeba *Acanthamoeba castellanii*

A. Saeed, H. Abd, B. Wretlind, G. Sandström (Stockholm, SE)

Objectives: *Pseudomonas aeruginosa* is a free-living, extracellular and a common environmental bacterium. It is an opportunistic and nosocomial pathogen causing serious health problems. To compete its predators such as macrophages and environmental phagocytes it utilises different survival strategies such as biofilm and formation of micro-colonies. It also develops resistance mechanisms and produces virulence factors such as lipopolysaccharide, alginate as well as extracellular, quorum sensing regulated enzymes and toxins and type III secretion system (TTSS). The aim of this study is to examine the interaction between *P. aeruginosa* PA103 and *Acanthamoeba castellanii*.
Methods: Bacteria and amoebae were co-culture and the interaction was examined between *P. aeruginosa* PA103 and *A. castellanii* by co-cultivation, viable count, eosin staining, electron microscopy, apoptosis assay and statistical analysis.
Results: The results showed that *P. aeruginosa* PA103 induced necrosis and apoptosis to kill *A. castellanii* by the effects of TTSS proteins ExoU, ExoS, ExoT, and ExoY. In comparison, growth of *Acanthamoeba* cultured alone and co-cultured with TTSS mutant strain was not affected.
Conclusions: The results confirm the nature of *P. aeruginosa* as a strict extracellular bacterium that needs TTSS to survive in the environment since this system is able to kill its eukaryotic predators such as Acanthamoebae.

O299 Triggering receptor expressed on myeloid cells-1 is involved in the pathogenesis of experimental sepsis by multidrug-resistant *Pseudomonas aeruginosa*

I. Tzepi, E. Spyridaki, C. Kranidioti, I. Vaki, V. Karagianni, H. Giamarellou, E. Giamarellos-Bourboulis (Athens, GR)

Objective: To define the contribution of TREM-1 (triggering receptor expressed on myeloid cells-1) in the pathogenesis of sepsis by multidrug-resistant *P. aeruginosa* (MDRPA).
Methods: An inoculum of $7 \log_{10}$ cfu/g of one MDRPA isolate was injected intraperitoneally in 35 B6C57 male mice. Survival was recorded in 10; the other mice were sacrificed at consecutive time intervals and blood was drawn from their inferior vena cava. Expression of TREM-1 was estimated after staining of white blood cells with anti-TREM-1 PE and flow cytometric analysis withgating for neutrophils. Tumour necrosis factor (TNF)-alpha and interleukin (IL)-6 were measured by ELISA.
Results: All animals died within 36 hours. Median expression of TREM-1 on neutrophils and median concentrations of TNFalpha and IL-6 after bacterial challenge are given in the Table.

Time (hrs)	TREM-1 (%)	TNF (pg/mL)	IL-6 (pg/mL)
2	19.00	37.39	288.43
3	16.15	NP	NP
4	17.85	39.31	222.87
5	21.17	NP	NP
6	48.47	31.25	145.74

NP: not performed.

Conclusions: Increase of the expression of TREM-1 on neutrophils is observed after that of pro-inflammatory cytokines. As a consequence, induction of TREM-1 might be an independent pathway in the pathogenesis of experimental sepsis by MDRPA.

Antibacterial susceptibility testing

O300 Evaluation of the Merlin MICRONAUT system for rapid direct susceptibility testing of Gram-positive cocci and Gram-negative bacilli from positive blood cultures

N. Wellinghausen, T. Pietzcker, S. Poppert, S. Belak, N. Fieser, M. Bartel, A. Essig (Ulm, DE)

Objectives: Bloodstream infections are life-threatening conditions which require timely initiation of appropriate antimicrobial therapy. We evaluated the automated Merlin MICRONAUT system for rapid direct microtiter broth antimicrobial susceptibility testing (AST) of Gram-positive cocci and Gram-negative bacilli from positive BACTEC 9240 blood culture bottles in comparison to the standard method on the Merlin MICRONAUT system.
Methods: This prospective study was conducted under routine working conditions during a 9-month period. Altogether, 504 isolates and 11,819 organism–antibiotic combinations from 409 patients were evaluable for comparison of direct and standard AST on the Merlin MICRONAUT system.
Results: Concerning Gram-negative bacilli, direct and standard AST was evaluated in 110 isolates and MIC agreement was found in 98.1% of 2,637 organism–antibiotic combinations. Category (SIR) agreement was found in 99.0%, with 0.04% very major, 0.2% major, and 0.8% minor errors. Concerning Gram-positive cocci, 373 isolates were evaluated and MIC agreement was found in 95.6% of 8,951 organism–antibiotic combinations. SIR agreement was found in 98.8%, with 0.3% very major, 0.4% major, and 0.5% minor errors. In addition, for the first time direct and standard AST were evaluated on 21 blood cultures positive with *Streptococcus* spp. MIC agreement and SIR agreement was found

in 96.5% and 97.8%, with 1.7% very major, 0% major, and 0.4% minor errors.
Conclusion: Direct AST of Gram-negative bacilli and Gram-positive cocci from positive blood cultures on the MICRONAUT system is a reliable technique that allows omitting repeat testing of subcultured isolates. Thereby, it significantly shortens the time to result of blood culture testing and has a positive impact on patient care.

O301 Detection of heteroresistant vancomycin intermediate *Staphylococcus aureus* in bloodstream infection

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Objectives: Poor clinical outcomes have been attributable to hVISA infection. Traditional methods using broth microdilution (BMD) seldom detect hVISA, and the optimal method to detect hVISA has not been clearly defined. We examined the prevalence of hVISA among 220 clinical *S. aureus* (SA) bloodstream isolates by Etest and population analysis profiles (PAP).
Methods: 220 SA bloodstream isolates from Buffalo VAMC (2003) were examined, which included 101 methicillin-susceptible SA (MSSA) and 119 methicillin-resistant SA (MRSA) isolates. SA strain ATCC 29213 was used as a control strain of vancomycin (VAN) susceptible. VAN MICs were determined by BMD according to CLSI. Etest was used as initial screening of hVISA, and PAP were performed for SA strains considered as hVISA. hVISA was defined as isolates which contained resistant subpopulations, but MICs of the parent strains were within the susceptible range (1–2 μg/mL). A modified starting inoculum was utilised (10^{10} CFU/mL) for PAP. Cultures were serially diluted and plated on BHI agar on VAN concentrations of 0.5–16 μg/mL (Wootton et al. JAC 48(1): 161).
Results: VAN MICs for 220 SA isolates ranged from 0.25 to 2 μg/mL in BMD; 12.3% (MIC ⩽ 0.5 μg/mL), 72.3% (MIC 1 μg/mL), 15.4% (MIC 2 μg/mL). MIC90 for MRSA = 2, MSSA = 1 μg/mL. Etest detected 13 isolates (5.9%) as hVISA, which were classified as susceptible per BMD. hVISA isolates were detected in 10.3% (10/97) of MRSA and in 2.44% (3/123) of MSSA (P = 0.014). All hVISA isolates detected by Etest were also classified as hVISA by PAP. Using PAP, 100% (13/13) of hVISA isolates displayed intermediately resistant subpopulations which grew on 4 μg/mL VAN BHI agar, 7.6% (1/13) grew on 6 μg/mL VAN BHI agar, and 0% on 8 or 16 μg/mL VAN BHI agar. Using a standard starting inoculum (10^8 CFU/mL) PAP detected only 11/13 (85%) hVISA isolates.
Conclusions: All SA strains were classified as VAN susceptible by BMD, but 5.9% of isolates were categorised as hVISA both by PAP and Etest. hVISA isolates were detected significantly more among MRSA than among MSSA. Starting inoculum was an important factor to detect resistant subpopulations. Our results suggest that Etest is a reliable method to screen for hVISA. This finding will have important implications on hVISA detection in clinical setting.

O302 New immunometric concept for rapid MRSA screening without pure-culture

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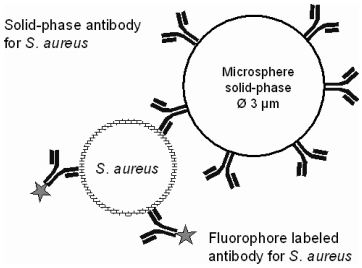
Objectives: Most of the MRSA (methicillin-resistant *Staphylococcus aureus*) screening methods currently in clinical use, including PCR, require pure culturing prior to the MRSA testing. The pure culturing causes two days delay between the sampling and the result. We present a new phenotypical technique for antimicrobial susceptibility testing. The new technique combines microbial culturing and specific immunometric detection in a single separation-free process, and allows on-line monitoring of microbe-specific growth.
Methods: A sample is incubated in growth medium, which contains antibody coated microspheres, fluorescent antibody reagent, and an antimicrobial agent in varying concentrations. As a result of the immunometric binding reaction, fluorescent cell-microsphere clusters are formed, which are then detected without separation steps by ArcDia TPX fluorescence detection technique (figure). The new MRSA screening technique applies dry-chemistry approach, where reagents are dispensed into the assay wells in the manufacturing process of the kits. Thus, only sample addition and incubation is needed prior to analysis. In order to demonstrate the performance of the technique for rapid MRSA screening several strains of Staphylococcus were tested.



Microspheres used as solid-phase reaction carriers for bioaffinity assay. Three-component immunocomplexes are formed on the solid-phase surface in proportion to the analyte concentration.

Results: The results show that the new technique enables antimicrobial susceptibility testing of MRSA in a time scale of 6–8 hours. Tolerance of the new technique to competing microbes was demonstrated by supplementing *S. aureus* samples with up to 400-fold excess of a competing microbe (*S. epidermidis*). Although competing-microbes lowered signal levels, the assay result remained the same. Presence of immunoreagents in the growth medium did not affect microbe growth.
Conclusion: In contrast to other genotypic or phenotypic methods, the new technique does not require pure culturing. This tolerance to competing microbes is a unique feature of the new technique. The results suggest that the new technique allows rapid detection of MRSA and antimicrobial susceptibility testing directly from clinical samples within 6–8 hours. This time is significantly shorter compared to methods that are currently in clinical use. Such a rapid and simple screening methodology would be valuable tool in clinical microbiology, since it shortens turn-around-times of microbiological analyses, and reduces empirical use of antibiotics. This improves cost-efficiency of antimicrobial resistance management.

O303 Evaluation of five commercially available rapid ESBL detection methods in a routine clinical microbiology laboratory

J. Lo-Ten-Foe, M. Hendriks-Franssen, A. Buiting (Tilburg, NL)

Objectives: We evaluated five commercially available easy-to-perform phenotypic tests designed for ESBL detection in our routine clinical microbiology laboratory.
Methods: We compared the E-test (Biotest), two different disk diffusion systems (supplied by Becton Dickinson and Oxoid), VITEK 2 susceptibility testing cards (AST-N020, AST-N048) lacking an ESBL test in combination with the Advanced Expert System (bioMérieux), and VITEK 2 susceptibility testing cards (AST-N041, EXT-N04) containing an ESBL test (bioMérieux). Both clinical isolates (*Escherichia coli* and *Klebsiella* species) from our laboratory and genotypically characterised ESBL-producing isolates were tested using the different phenotypic testing methods.
Results: A high sensitivity (87.5–100%) was observed for all tested phenotypic ESBL detection methods in the clinical isolates and in the genotypically characterised ESBL-producing isolates. The specificity for the two disk diffusion systems, E-test and VITEK 2 susceptibility testing cards containing an ESBL test was also high (80–100%). However, the specificity for the VITEK 2 susceptibility testing cards lacking an ESBL test was low (21–25%).
Conclusion: VITEK 2 susceptibility testing cards lacking an ESBL test are suitable for initial screening for ESBL production since they display high sensitivity. The E-test and the two tested disk diffusion methods

reliably detect ESBL-producing isolates. In general, the E-test and the disk diffusion methods are used to confirm ESBL production in a specific isolate. More time is required to perform these extra confirmation tests following initial screening tests for ESBL production. However, the ESBL test containing VITEK 2 susceptibility testing cards make it possible to do both the screening and the confirmation in one test, allowing for faster and reliable ESBL detection.

O304 What system for the glycopeptides and oxazolidinone susceptibility testing among *Enterococcus faecium* isolates?

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Objectives: The performances of three widely diffused, commercially available, methods for the susceptibility testing of glycopeptides and of oxazolinidone were compared against clinical isolates of *E. faecium*
Methods: Thirty strains of *E. faecium* were consecutively isolated from clinical specimens of patients hospitalised during the year 2005 at the "Azienda Policlinico Umberto I" Academic Hospital of Rome, Italy. Strain identification was performed with both VITEK 2® and Phoenix® automated systems. The antimicrobial susceptibility testing for teicoplanin, vancomycin, and linezolid, was performed by the VITEK 2, by the Phoenix and by the E-test® method, according to the manufacturers' instructions. Detection of 23S rRNA gene G2576T mutation was carried out by PCR-RFLP using specific primers.
Results: The automated systems fully agreed in the strain identification. MIC values for teicoplanin $\geqslant$16 ug/mL were detected in 6, 16, and 17 isolates by the VITEK 2, the Phoenix, and the E-test respectively. Furthermore, differences >2 twofold dilutions were observed for 11 strains between the VITEK 2 and the Phoenix, for 17 strains between VITEK 2 and E-test, and for three strains between Phoenix and E-test. For vancomycin, both VITEK 2 and Phoenix evidenced MIC values $\geqslant$16 ug/mL in 25 strains, agreed in three susceptible strains, while in two cases they showed discordant results. The E-test showed MIC values for vancomycin $\geqslant$16 ug/mL in 22 strains, in six isolates displayed results discordant with the automated systems, while agreed in two cases of susceptible strains. In all instances but one, the three methods agreed in the susceptibility testing for linezolid. The only strain carrying 23S rRNA mutation showed MIC values for linezolid $\leqslant$4 ug/mL when tested with the Phoenix and with the E-test, while with the VITEK 2 displayed a MIC > 8 ug/mL.
Conclusions: Glycopeptide resistance of Enterococci poses serious therapeutic problems and an accurate microbiological diagnosis is fundamental for the treatment. Our results evidenced a high rate of discrepancies in the detection of glycopeptide resistance by three widely diffused susceptibility testing methods. Furthermore, some limits were observed in the detection of linezolid resistance that prospectively could reduce the therapeutic options.

The emerging β-lactam resistance in *Escherichia coli*

S309 Emerging broad-spectrum β-lactamases in *Escherichia coli*: latest ESBLs and carbapenemases

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Increasing β-lactam resistance in *E. coli* has become a worrying threat worldwide. In that species, most of the mechanisms involved in the β-lactam resistance are linked to the production of β-lactamases, including clavulanic-acid inhibited expanded-spectrum β-lactamases (ESBLs) and carbapenemases which are the most powerful enzymes able to degradate most β-lactams. Besides the most common ESBLs circulating in Enterobacteriaceae (TEM-, SHV, and CTX-M-types), there are other emerging enzymes which are mostly plasmid-encoded and are distributed in a large variety of species. The ESBL VEB-1 is known to be largerly distributed in South-East Asia, PER-1 in Turkey, Italy, Korea, and also South America, and GES/IBC-type enzymes in Greece and Japan mostly. All these ESBLs are known to efficiently hydrolyze expanded-spectrum β-lactams, and some variants of the GES family are also hydrolysing imipenem at a low level. Other enzymes of the KPC family have been also reported in *E. coli*. KPC-2 has been shown to be prevalent in *E. coli* in Israel and KPC-3 has been reportyed sporadically in the USA. These latter enzymes are considered as ESBLs since they are inhibited by clavulanic acid and their hydrolytic efficiencies toward imipenem are very high, thus giving rise to high level resistance to carbapenems in those *E. coli* isolates.
In addition to these ESBL determinants, metallo-β-lactamases (MBL) have been identified in *E. coli*. These enzymes which are not inhibited by clavulanic acid and hydrolyse carbapenems very efficiently are of the IMP and VIM groups. In *E. coli*, IMP-1 has been identified in Japan and VIM-2 in Greece, but the most worrying observation is that related to the spread of VIM-1 in Greece. Indeed, this determinant has been identified in clinical isolates also harbouring ESBL encoding genes, thus leading to panresistance in those strains.
Noteworthy, most of the ESBL and MBL encoding genes encountered in *E. coli* are vehiculed by plasmids and located into class 1 integron structures, thus facilitating the occurrence of multidrug resistance via the co-localisation of other antibiotic resistance genes.
A particular attention will be needed in the next future to trace the isolates harbouring these worrying determinants, and also to trace the corresponding plasmids which are powerful tools for such dissemination.

Surrogate markers of disease severity and for guidance of antimicrobial therapy

S311 Monitoring severe sepsis and therapy

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Severe sepsis is a serious syndrome with organ failure that may affect a large proportion of the patients admitted to the intensive care unit and whose prediction is often difficult [1]. The Surviving Sepsis Campaign (SSC) is a global programme to reduce mortality rates in severe sepsis, endorsed by 11 International Medical Societies. This Campaign produced the guidelines for management of severe sepsis and septic shock [2]. These recommendations were reviewed during the annual meeting of the Society of Critical Care Medicine held in January 2006.
The Campaign has taken an action to create National and International networks to collect data and monitor interventions. In Italy the network was settled in September 2006. But, can we monitor therapy for severe sepsis if concepts, tools, ideas and attitudes differ largely between centres? With the aim of answering this crucial question, we conducted a survey on the perception of Severe Sepsis, its diagnosis and monitoring through the national network for the Italian Chapter of the SSC.
Twenty-three participating centres (10 community and 13 teaching hospitals) responded to our survey, for a total number of 261 ICU/critical care beds. When the participants were asked to describe the methodology currently used for implementation of data in their institution, 70% of the respondents adopted a continuous data collection methodology and the other 30% a "before and after event" design.
On the question whether an educational tool was used to sensitise people to the campaign, 13% of the respondents declared that no specific tool was used and the other 87% (20 centres) stated that they used different educative means (mostly chart documentations and department conferences).
When asked when data collection for the diagnosis of sepsis usually takes place, thirteen respondents said that their collection occurred within 24 hours from the diagnosis, only 2 contemporary, and 8 retrospectively. On the question how data were collected 9 institutions out of 23 (39%) answered that the SSC paper tool or database were used, 11 (47%) a combination of the two, and 3 (23%) other systems.
In 17 of the 23 centres the responsibility of data collection is assigned to the attending physician; in the remaining 5 cases residents, nurses or students collect the data, but same centres allow the simultaneous collaboration of more figures.

Partnership between ICUs and other departments to approach Sepsis in the spirit of the SSC is unfortunately scarce: only 4 (17%) of the respondents have a form of collaboration with other units. The effect of this mentality is that only 6 (26%) of the interviewed hospitals currently apply a screening for severe sepsis in the ward.
One main concept that illustrates the spirit of the SSC is the idea of an early intervention to apply the different recommendations. Again, in checking how the time of presentation of sepsis is conceived in the emergency department, in the ward and in the ICU we obtained answers that were quite diverse. Of the 23 participating institutions, 16 responded. Only 50% of physicians in the Emergency Department identified the "presentation time" of severe sepsis with the moment of triage or hospital admission. The remaining 50% had a sort of "a posteriori" diagnosis, losing the possibility of an early approach. Things are much worse looking at the ward, where in 80% of cases the moment of severe sepsis presentation corresponded to the identification of symptoms from the "a posteriori" reviews of the medical notes, the arrival of a critical care consultant or ICU admission. Surprisingly, in the ICU still 30% of the cases is usually diagnosed through the review of the medical reports.
Conclusions: At present, despite the tremendous effort made by the International Intensive Care Community to standardise the therapy for severe sepsis, predicating the comandements of the SSC and boundles, a large variability exists in the concept of time, tool availability, and logistics.

Reference(s)

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Fungal diagnostics

S312 Antifungal susceptibility testing

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The EUCAST Subcommittee on Antifungal Susceptibility Testing (EUCAST-AFST) has recently published a standard for determining the susceptibility of fermentative yeasts to antifungals. Besides, another standard related with the susceptibility of filamentous fungi to antifungals will be published very soon. The EUCAST General Committee has recently published guidelines for harmonisation of breakpoints for antimicrobials and therefore the Antifungal Susceptibility Testing Subcommittee of EUCAST has adopted it. In order, to prove that breakpoints for antifungals can be obtained, EUCAST-AFST decided to make a proof of concept with fluconazole. All the requirements raised for EUCAST committee to obtain break points for antimicrobials have been addressed and fluconazole break points have been attained. The data obtained through the revision system to obtain break points for fluconazole will be discussed. Also the discrepancies of EUCAST and CLSI break points will be examined and analysed. In the near future, it is intention of EUCAST AFST develop break points to all licensed antifungals.

Epidemiology of nosocomial infections in the ICU

O318 Bloodstream infections due to *Klebsiella pneumoniae* in an intensive care unit in Greece: susceptibility patterns and clinical characteristics

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Objectives: In recent years *K. pneumoniae* has been shown to have increasing resistance, due to production of extended-spectrum β-lactamases (ESBLs) or metallo-β-lactamases (MBLs). The aim of this study was to record the bacteraemias due to *K. pneumoniae* in our ICU, to analyse the patterns of resistance, as well as the clinical characteristics of the ensuing infections.
Methods: Prospective observational study in patients who were hospitalised in the ICU, from September 2004 to October 2006. The demographic characteristics of all patients admitted were recorded, as well as the underlying diseases, disease severity as estimated by the APACHE II score and possible factors predisposing to infections, such as previous consumption of antibiotics. Susceptibility testing of culture isolates was performed by MicroScan autoSCAN4. The outcome of all bacteraemias was recorded. Statistical analysis was done with ESPSS v.12.
Results: During the study period 290 patients were admitted to the ICU and 160 episodes of bacteraemia were recorded. Of these, 29 (18%) were due to *K. pneumoniae*. The isolates were resistant as follows: 100% to ampicillin, 72% to β-lactamase inhibitors, 90% to 3rd-generation cephalosporins, 41% to imipenem (MIC > 4 mg/L), 41% to aztreonam, 86% to quinolones and 31% to aminoglycosides. All isolates were susceptible to colistin (MIC ⩽ 2 mg/L). The carbapenem-resistant isolates (CRKP) were also resistant to all other antibiotics tested, except aztreonam and gentamicin (75% and 58% sensitive respectively). The bacteria were isolated in 23 patients (mean age 67 years, 69% male) of whom 56% were surgical. Their mean APACHE II score was 19. Ten bacteraemias were primary, 5 secondary to ventilator-associated pneumonia and 14 catheter-related. The 14-days mortality of patients with bacteraemia due to CRKP was 53%, while in those with sensitive strains it was 28% ($p = 0.2$). Outcome was significantly related to the APACHE II score ($p = 0.02$). Of the patients who had bacteraemia due to CRKP, 77% had been previously receiving a carbapenem. Of those who had a carbapenem-sensitive isolate, 35% had been receiving a carbapenem ($p = 0.4$)
Conclusions: Infections due to *K. pneumoniae* resistant to carbapenems represent a serious clinical problem in our ICU and are associated with a high mortality rate. It seems that previous use of carbapenems leads to increased resistance. Larger studies are needed in order to obtain statistically significant results.

O319 Epidemiologic surveillance of nosocomial infections in the intensive care unit: role of risk factors, emerging pathogens and cross-transmission

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Objectives: Nosocomial infections surveillance was performed in an Italian ICU in order to pretest the HELICS protocol before its nationwide implementation, and to evaluate the impact and the routes of acquisition of three emerging multiresistant pathogens, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Stenotrophomonas maltophilia* by determining: (i) the occurrence of carriage on admission; (ii) the microrganism-specific ICU-acquired infection rates, by site; (iii) the impact of cross-transmission using molecular typing data, and (iv) the individual risk factors for infection.
Methods: A six-months surveillance survey was performed at the ICU of an Italian Hospital, in accordance with the HELICS protocol, excluding patients staying less than three days. An electronic data form, was designed using the SPSS Data Entry Enterprise Server (SPSS Inc.). Clonality was determined by macrorestriction analysis using well established criteria, and the presence of two indistinguishable strains in two patients was considered one episode of cross-transmission.
Results: During the study period, 121 patients were enrolled into the survey for a total of 2165 days. The ICU-acquired infection rate was 82.6% patients and the incidence density was 46.2‰ patient-days. The occurrence of *P. aeruginosa* carriage on admission was 1.6% patients. No episode of *A. baumannii* or *S. maltophilia* carriage was identified. The ICU-acquired *P. aeruginosa*, *A. baumannii* and *S. maltophilia* associated infection rates were respectively: 35.9%, 13.0% and 12.4% patients.

The incidence density were respectively 19.4‰, 7.4‰, 6.9‰ patient-days. ICU-acquired pneumonia was confirmed to be the first infection type (30.0%), followed by bloodstream infections (BSIs) (25.0%), local CVC-related infections (23.0%), urinary tract infections (13.0%), CVC-related BSI (8.0%), and surgical site infections (1.0%). Eighteen *P. aeruginosa*, one *A. baumannii* and four *S. maltophilia* distinct clones were identified by macrorestriction analysis over a total of 162 isolates. The impact of cross-transmission was estimated to be at least 52.4%, thus defining the preventable proportion of all cross-transmission episodes. Two major risk factors were identified: inappropriate management of invasive devices and of antimicrobial usage.

Conclusion: Our study confirms the essential role of epidemiologic surveillance to provide advanced risk-adjusted infection rates as a measure of quality of care.

O320 European surveillance of ICU-acquired infections (HELICS-ICU), 2004–2005: ICU-acquired pneumonia

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Objective: To describe the 2004–2005 results of the European surveillance of ICU-acquired pneumonia in the HELICS-ICU (HI) network (http://helics.univ-lyon1.fr).

Methods: Six patient-based networks (Austria, Belgium, France, Spain, Luxemburg and Lithuania), 2 piloting countries (Norway, Slovakia) and 1 unit-based (Germany) surveillance networks contributed data on 15,161 episodes of ICU-acquired pneumonia (PN) from 721 ICUs between Jan 2004 and Dec 2005. Since the HI protocol excludes patients with a length of stay (LOS) of <3 days in the ICU, data from Germany (no denominator data for patients staying >2 days) were excluded for the calculation of indicators, but included for the description of infections.

Table 1. Relative frequency of 10 most frequent isolated micro-organisms in ICU-acquired pneumonia, HELICS-ICU, 2004–2005

	AT	BE	DE	ES	FR	LT	LU	Total
No. of ICUs	43	33	329	112	185	12	7	721
No. of isolates in PN	2087	1587	6074	1279	4385	97	58	15,567
S. aureus	12.8%	12.0%	21.9%	20.4%	22.4%	17.5%	8.6%	19.6%
%MRSA/SA	38.8%	38.2%	34.5%	38.4%	44.8%	NA	NA	38.7%
P. aeruginosa	22.2%	18.8%	14.6%	17.7%	23.0%	23.7%	22.4%	18.8%
Escherichia coli	6.4%	8.8%	9.9%	6.4%	8.1%	3.1%	12.1%	8.5%
Klebsiella spp.	7.7%	7.6%	10.7%	6.4%	5.6%	2.1%	10.3%	8.1%
Enterobacter spp.	6.5%	11.7%	7.9%	5.6%	6.7%	1.0%	8.6%	7.5%
Candida spp.	12.5%	3.3%	4.8%	2.3%	2.5%	0.0%	5.2%	4.8%
Haemophilus spp.	2.4%	5.6%	3.3%	6.4%	5.3%	14.4%	3.4%	4.3%
Enterococcus spp.	7.4%	1.6%	5.0%	1.6%	1.0%	1.0%	5.2%	3.6%
Streptococcus spp.	3.2%	2.8%	2.2%	3.8%	5.6%	9.3%	0.0%	3.5%
Acinetobacter spp.	3.1%	1.1%	2.5%	10.2%	3.1%	14.4%	0.0%	3.3%

Results: Of 87,353 patients staying more than 2 days in the ICU, 8.7% acquired a PN (intubator-associated 89.9%). The median incidence density varied from 3.3 PN episodes per 1000 patient-days (pd) (distribution ICUs P25 0.0; P75 7.7) in ICUs with less than 30% patients intubated, to 6.4 (P25 3.1; P75 10.4) in ICUs with 30–59% patients intubated and 9.4 (P25 4.8; P75 13.9) in ICUs with ⩾60% of patients intubated. The incidence was higher in polyvalent ICUs (P50 7.6 PN/1000 patient-days) than in surgical (P50 4.4) and medical (P50 5.7) ICUs. The most frequently reported micro-organism was *S. aureus* (19.6%) with an average MRSA/SA percentage resistance of 38.7%. There were marked differences in the relative frequency of isolated micro-organisms between countries (table).

The diagnosis of PN was confirmed by quantitative culture (HELICS definition PN1 or PN2) in 79% in FR, 54% in ES, 32% in AT, 21% in BE, 15% in LT and 7% in LU. In the piloting countries (limited numbers), 71% was confirmed in Norway and 0% in Slovakia.

Conclusion: Although underlying differences in diagnostic practices persist, the compatibility with the HI protocol is increasing. The level 1 (unit-based) data presented here provide sufficient indicators for continuous follow-up of infection rates within the ICU and limited risk-adjusted inter-ICU comparisons with a low workload.

O321 REA-RAISIN: National surveillance network of ICU-acquired infections, France, 2005

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Background: The objective of nosocomial infections (NI) surveillance in intensive care units (ICU) is to assess and compare rates over time and amongst ICUs, in order to provide an evidence-based approach for improving infection control practices.

Methods: In France, regional ICU surveillance networks have been implemented since 1994. In 2004, surveillance methods were standardised through a national surveillance project (RAISIN). The national REA-RAISIN network conducts 6 months a year a patient-based NI surveillance in ICU. On a voluntary basis and using a standardised methodology, participating ICUs collect data for each patient staying more than 2 days in the unit. Surveillance focuses on device related-infections, providing the units with incidence rates of ventilator-associated pneumonia (PNE), urinary tract infections (UTI), central venous catheter colonisation (COL) with or without catheter-associated infections (CRI) and nosocomial bacteraemia (BAC).

Results: From January to June 2005, 151 ICUs included 20,632 patients.

- Patient characteristics: mean age 61.7 yrs, gender ratio 1.62, type of admission (medical 68%, scheduled surgical 17%, emergency surgical 15%), trauma 9%, impaired immunity 12%, patient origin (home 54%, acute care 38%, LTCF 4%, other ICU 3%), antibiotic treatment at admission 52%, mean SAPS II 40.1, mean length of stay 11.2 days.
- Exposure to invasive devices: intubation/tracheotomy 60.5%, central venous catheter (CVC) 57.6% and indwelling urinary catheter (UC) 79.2% (device utilisation ratio = 58.1%, 62.0% and 74.2% respectively).
- A total of 5,159 nosocomial events were documented concerning 2,569 patients (12.4%).
- Overall incidence rates were calculated: 17.6 PNE/1,000 intubation-days, 5.5 COL/1,000 CVC-days (or 2.2 CRI/1,000 CVC-days), 3.3 BAC/1,000 hosp.-days and 7.9 UTI/1,000 UC-days.
- Unit distributions were performed with great variations concerning patient/unit characteristics and rates. Further analyses are planned, using a multivariate model (standardised incidence ratios) in order to improve benchmarking possibilities.

Conclusion: This report on NI surveillance in ICU from a large sample of French hospitals will serve as a national reference and will allow describing, evaluating and monitoring NI in ICUs. Feedback to ICUs provides them with relevant information to monitor and target infection control policies.

http://www.invs.sante.fr/raisin/

O322 Nosocomial invasive pneumococcal disease in Toronto, Canada, 1995–2005

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Background: Recently, several outbreaks of antibiotic resistant nosocomial invasive pneumococcal disease (IPD) have been reported. We review the occurrence of nosocomial IPD in Toronto, Canada from 1995 to 2006.

Methods: Population based surveillance for IPD in residents of metropolitan Toronto and Peel region, Ontario, Canada (population 3.9M) has been on-going since 1995, and surveillance for disease associated with respiratory isolates of *S. pneumoniae* (SPN) since 2002. Nosocomial disease is defined using US NNISS criteria.

Results: From 1995 to 2005, 4,836 episodes of IPD have been identified of which 208 were nosocomially acquired. The overall average incidence of nosocomial IPD was 0.53/100,000/y. There was no change in incidence over time although the incidence of community-acquired IPD decreased from 13.5 to 7.9/100,000/y during the same period. The median age of patients was 68 yr (range 0.12–97 yr); 34 (16%) were children. 125 (60%) were male, 179 (86%) had chronic illness qualifying them for receipt of pneumococcal vaccine [most commonly: cancer (80, 38%), cardiac disease (73, 35%), lung disease (50, 24%), diabetes mellitus (48, 23%)]. The most common serotypes causing disease were 6B (21), 23F (20), 6A (16) and 22F (14). Overall resistance was: erythromycin 13.6%, penicillin 5.7%, ceftriaxone 2.5%, levofloxacin 3.7%, moxifloxacin 0%. From 2002 to 2006, 391 patients with respiratory isolates of SPN were identified; 103 episodes met criteria for non-bacteraemic pneumococcal pneumonia (NBPP). Median age of patients was 65 yr, 73% were male, 83% had a qualifying chronic underlying illness. The most common serotypes were 19F (67), 6A (31), 6B (25), and 3 (24). Resistance rates were: erythromycin 21%, penicillin 6.3%, ceftriaxone 4.2%, levofloxacin 1.8%, moxifloxacin 1.1%. The case fatality rate was 29% in NBPP, and 45% in IPD. In IPD episodes, levofloxacin resistance (LEVR) increased from 0 in 1995/8 to 9.5% in 2001 ($P = 0.25$). From 2002 to 2005, LEVR decreased in both IPD and non-invasive isolates, from 8.8% (2001) to 1.4% (2006) ($P = 0.01$). Examination of cases for clustering in time and space revealed clusters of 2–5 cases each comprising 38/599 (6.3%) cases.

Conclusion: Nosocomial pneumococcal disease is uncommon, but associated with a high case fatality rate. Most disease is sporadic. In Toronto, levofloxacin resistance is decreasing in nosocomial isolates while remaining stable in the community: this may be due to changes in hospital antibiotic use.

Mycobacterium tuberculosis: cell biology and molecular pathogenesis

O323 Execution of macrophage apoptosis by PE_PGRS33 of *Mycobacterium tuberculosis*

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Objectives: The role of the multi-gene PE family of proteins unique to mycobacteria, in the pathogenesis of tuberculosis is still poorly understood. Tumour necrosis factor-alpha (TNF-α) is essential for successfully combatting tuberculosis. Our objective was to understand how PE_PGRS33, a surface-exposed protein known to undergo variation among strains, influences TNF-α release from macrophages, and how this is linked to apoptosis of macrophages.

Methods: Recombinant PE_PGRS33 was tested for its ability to release TNF-α from the macrophage cell line RAW264.7 as well as from human monocyte-derived macrophages. Its binding to cell surface TLR2 was analysed by flow cytometry and the role of TLR2 was studied by transfecting cells with dominant-negative TLR2 prior to analysing PE_PGRS33-induced TNF-α release. The signaling pathways activated were studied by assaying for the mitogen-activated protein kinase (MAPK) kinase kinase ASK1 and downstream MAPKs. Cell death was analysed using histone ELISA and also by assaying surface-associated annexin-FITC.

Results: PE_PGRS33, a surface exposed protein, elicits TNF-α release from macrophages in a Toll-like receptor 2 (TLR2)-dependent manner. Apoptosis signal-regulating kinase 1 (ASK1) is activated downstream of TLR2. ASK1 activates the mitogen-activated protein kinases (MAPKs) p38 and JNK. PE_PGRS33-induced signaling leads to enhanced expression of TNF-α and TNF receptor I (TNFRI) genes. Mycobacterium smegmatis expressing PE_PGRS33 elicits the same effects as purified PE_PGRS33. Neutralising TNF-α antibodies showed that release of TNF-α is required for triggering apoptosis in macrophages challenged with PE_PGRS33. The death receptor-dependent signals are amplified through classical caspase 8-dependent mitochondrial release of cytochrome c, leading to the activation of caspases 9 and 3.

Conclusions: The important aspect of our findings is that deletions within the PGRS domain (simulating those occurring in clinical strains) attenuate the TNF-α-inducing ability of PE_PGRS33. These results provide the first evidence that variations in the polymorphic repeats of the PGRS domain modulate the innate immune response.

O324 Cytokine secretion, phagosomal maturation and killing capacity of human neutrophils is differentially influenced by virulent and avirulent strains of *Mycobacterium avium*

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Objective: Polymorphonuclear neutrophils (PMNs) have been implicated in the early inflammatory response against mycobacteria besides monocytes/macrophages. Whereas virulent mycobacteria manage to survive inside macrophages (MΦ) for a prolonged period of time by blocking the phagosome from fusion with late endosomes and lysosomes, little is known about the potential of mycobacteria to alter the basic biologic behaviour of PMNs. We studied the influence of virulence of different *M. avium* strains on killing capacity, cytokine production and phagosomal maturation of PMNs.

Methods: PMNs isolated from 20 healthy donors were infected with *M. avium* (2 virulent and 2 avirulent strains) and incubated at 37ºC for defined time periods. Neutrophil killing activity: dilution series of released intracellular bacteria (SDS lysis) were plated on 7H10 agar. Colony forming units (CFUs) were counted after 7 days. Cytokines in the supernatant were measured by ELISA. FITC labeled *M. avium* were simultaneously visualised with Alexa-Fluor® antibody staining of early endosomal and lysosomal markers respectively (Rab5 and LAMP-1) by fluorescence microscopy. Statistical significance of data was calculated by Student's t-test except CFU-values and killing index respectively were analysed by variance analysis. Post hoc pairwise comparisons were done using Scheffes' test.

Results: At 2 h 53.73±7.73% of virulent *M. avium* initially phagocytised were killed, whereas killing was virtually complete, 96.58±0.71%, for avirulent strains. IL-8 and MIP-1a in supernatants of PMNs infected with avirulent *M. avium* strains were significantly higher ($p = 0.004$) than in PMNs infected with virulent *M. avium* strains. IL-1b secretion of PMNs was independent of virulence of *M. avium*. However the receptor antagonist of IL-1, IL-1ra, was significantly higher in supernatants of PMNs infected with virulent strains. At 1 h 83.8±12.9% of the membranes of all cell-associated avirulent, but only 27±6.3% of virulent, *M. avium* strains stained positive for LAMP-1. In contrast 78.96±10.7% of virulent, but only 35.8±7.5% of avirulent, strains stained positive for rab5.

Conclusion: Virulent strains of *M. avium* escape early host defence by inhibition of proinflammatory cytokines and blocking of phagosome maturation of PMNs. Thus PMNs may serve as a trojan horse for virulent mycobacteria. The latter, once released from PMNs, are then capable of infecting new host cells such as macrophages.

O325 Characterisation of polyphosphate kinase 1 of mycobacteria and its role in persistence

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Objectives: Polyphosphate kinase (PPK1) is the principal enzyme required for the synthesis of inorganic polyphosphate (polyP), a polymer of tens or hundreds of Pi residues linked by high energy phosphoanhydride bonds. It plays an important role in long term bacterial survival in stationary phase, against nutritional, oxidative, osmotic and thermal stress, and is associated with virulence of several pathogens. *Mycobacterium tuberculosis*, the causative agent of tuberculosis, survives inside host macrophages in a nutritionally starved and stressed condition.

Our objective was to understand the role of PPK1 in bacterial survival under stress.
Methods: To evaluate the role of PPK1 in mycobacterial survival, the gene encoding PPK1 was disrupted in M. smegmatis through homologous recombination. The effect of stress on the wild type and the mutant were then analysed. ppk1 was also silenced by antisensing in *M. tuberculosis*.
Results: The PPK1-deleted strain of M. smegmatis exhibited significantly reduced intracellular survival in murine RAW 264.7 macrophages compared to the survival of its wild type counterpart. It exhibited an extended lag phase of growth when shifted to a low phosphate-containing medium, was unable to grow in nitrogen-depleted medium and impaired in its ability to survive in anaerobic conditions. It showed decreased transcription of the stringent response regulator relA. *M. tuberculosis* ppk1 could complement the mutant. Antisensing of the ppk1 gene in *M. tuberculosis* also showed reduced levels of relA transcription. The ppk1 mutant was defective in its ability to form biofilms.
Conclusion: ppk1 likely plays a role in mycobacterial persistence and is a potential target for drug development.

O326 Cholesterol oxidase as a virulence factor of *Mycobacterium tuberculosis*

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Objectives: The objective of this study was to evaluate cholesterol oxidase (ChoD) as a putative virulence factor of *Mycobacterium tuberculosis*.
Methods: A two-step recombination protocol was used to replace the cholesterol oxidase gene with a non-functional copy. The pathogenicity of resultant strains was analysed in vitro using peritoneal macrophages and in vivo by mice infection.
Results: Cholesterol oxidase is known to be a key enzyme initiating cholesterol degradation processes in many soil bacteria, including fast growing mycobacteria. Pathogenic mycobacteria accumulate cholesterol without its utilisation and express cholesterol oxidase as an extracellular enzyme. A homologous recombination was used to construct an *M. tuberculosis* strain with an unmarked deletion within the choD gene. The wild type *M. tuberculosis* strain (Mt-wt), choD-mutant (mut-choD) and mutant complemented with choD gene controlled by a heat shock protein promoter (mut-choD-PhspchoD) were used to study the function of cholesterol oxidase in mycobacterial pathogenesis. Peritoneal macrophages (10^6/well) were infected with Mt-wt, mut-choD and mut-choD-PhspchoD *M. tuberculosis* strains (10^5/well) and incubated for four and six days. The numbers of Mt-wt and mut-choD-PhspchoD viable bacteria recovered from macrophages were at least an order of magnitude higher compared to mut-cho strain as revealed by cfu analysis. The same strains were used for infection of mice. Ten weeks post infection, lungs and spleens were isolated from euthanised mice. The numbers of viable bacteria in each organ were detected by cfu. Analysing at least 10 mice of each group, the strong attenuation of choD mutants was observed compared to wild type and complementation strains.
Conclusion: Cholesterol oxidase is an important virulence factor during infection by *M. tuberculosis*.
This work was supported with KBN grant: contract no. 3P05A14024

O327 Ex vivo profiling of cytokines/chemokines in patients with tuberculosis and latent tuberculosis infection

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Objectives: While in most cases infection with *Mycobacterium tuberculosis* (Mtb) is contained (latent Mtb infection; LTBI), it progresses to overt disease (TB) in a small proportion of those infected. We studied the cytokine/chemokine profiles associated with LTBI and TB in order to gain insight into ongoing immune activation events in Mtb-infection.
Methods: Blood was obtained from patients with culture-confirmed TB (n = 4), with recently acquired LTBI (n = 3), and from healthcare workers without Mtb exposure (n = 6). Interferon-gamma (IFN-γ) that was released from sensitised lymphocytes upon ex vivo stimulation with Mtb-specific antigens was determined using the QuantiFERON-TB® Gold In Tube assay as recommended by the manufacturer (Cellestis Ltd., Carnegie, Australia). Microsphere-based multiplex assays (Lincoplex®, Linco Research Inc., St Charles, MA, USA) were used to assess the plasma concentrations of IFN-γ, TNF-α, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p40, IL-12p70 IL-13, IL-15, Fraktalkine, GM-CSF, MCP-1, and IP-10 before (NIL) and after (TbAg) ex vivo stimulation. Values were determined using Bio-Plex Manager (Bio-Rad Laboratories, Hercules, CA, USA). The limit of detection was 3.2 pg/mL. Statistical analysis was performed using Prism 4.1 (GraphPad Software Inc., San Diego, CA, USA). The Kruskal-Wallis test was used for multiple group comparison, and the Mann-Whitney test for group to group comparison.
Results: IFN-γ, IP-10, IL-2, GM-CSF, IL-13 concentrations were significantly higher in patients with TB and LTBI as compared to controls (Table).

	ΔTb Ag – NIL, median (range), pg/mL]			ANOVA	Mann–Whitney	
	Control	TB	LTBI	Kruskal-Wallis	Control vs TB	Control vs LTBI
IFN-γ	−0.2[−3.0 – 2.0]	175.3 [131.0 – 238.6]	115.9 [2.2 – 185.4]	P < 0.02	P < 0.03	P < 0.03
IP-10	212.0 [126.0 – 1409]	18,188 [3,806 – 46,279]	12,996 [1,809 – 24,112]	P < 0.02	P < 0.01	P < 0.03
IL-2	0.47 [−4.6 – 59.9]	630.5 [404.5 – 5,871]	413.5 [280.1 – 887.5]	P < 0.02	P < 0.01	P < 0.03
GM-CSF	−2.3 [−12.9 – 11.0]	27.6 [2.9 – 481.2]	20.8 [16.6 – 59.1]	P < 0.02	P < 0.02	P < 0.03
IL-13	−2.8 [−34.6 – 14.2]	61.6 [0.2 – 144.2]	57.6 [2.9 – 59.5]	P < 0.04	P < 0.04	P < 0.05

Conclusions: Mean plasma concentrations of IP-10 were 50 to 90-fold higher than those of IFN-γ, both in patients with active TB and LTBI. Assessment of IP-10 might substantially increase the sensitivity of IFN-γ release assays (IGRA) for detecting Mtb infection. Ex vivo profiling of cytokines/chemokines using IGRA in combination with flow-cytometer multiplex assays identified additional candidate parameters to confirm Mtb infection and will improve our understanding of TB pathogenesis.

Clostridium difficile

O328 Community rather than hospital factors promote increase of *Clostridium difficile* associated diseases in Northern Bavaria

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Objectives: Recently, the number of *C. difficile* associated diseases (CDAD) has increased. Prevalence data of CDAD particularly resulted from analyses performed at university hospitals, etc. In our lab we perform microbiological analyses for more than 3500 facilities comprising more than 40 hospitals in Northern Bavaria allowing examination of *C. difficile* prevalence in the local region.
Methods: Stool samples were analysed by ELISA detecting toxins A and B of *C. difficile* (Cdt). The total number of ELISA performed in 2004, 2005, and 2006 and the number of positive results was determined by hybase analysis. All *C. difficile* positive patients identified in 2005 were documented. Analysis of toxin genes IStron types integrated into the genome (*C. difficile* integration profiles) was performed on *C. difficile* cultured from stool samples of 17 patients treated at 2 hospitals.
Results: The number of Cdt positive stools samples increased from 372 in 2004 to 515 (2005) and 642 (Jan–Sept 2006). From October 2005 to January 2006 there was a marked increase from 31 to 103 Cdt positive stool samples per month. High numbers were also observed in the subsequent period until June 2006 (median 84/month). In July this number decreased to 39. When this analysis was performed on Cdt positive findings of patients from 4 harvested hospitals (I, II, III, IV) courses of each facility was very similar to that of the total collective suggesting that increase of CDAD in particular depended on community factors and secondarily on hygiene of certain hospitals. Typing analysis

results suggested that all isolates from patients of hospitals I and II were identical. No transmissions of patients from hospital I to hospital II had been found and vice versa indicating prevalence of one prevailing *C. difficile* strain in the local region. Binary toxin was not found in these isolates indicating the strain neither NAP 1 nor 027.
Conclusion: One *C. difficile* strain seems to be predominating in Northern Bavaria CDAD patients. The increased number of CDAD cases was probably promoted by common events, e.g. increased antibiotic use due respiratory infects in winter. Therefore, infection control programmes to restrict spread of *C. difficile* should not be restricted to hospitals but also should imply other healthcare facilities (nursing homes, house doctors).

O329 High incidence of *Clostridium difficile* associated diarrhoea with a community onset in a hyperendemic region in Germany

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Background: Recent studies from UK and USA suggest an increase of community-acquired CDAD, possible associated with use of proton pump inhibitors (PPI).
Objectives: To investigate the incidence of CDAD among patients with diarrhoea visiting the general practitioner and using the proposed definitions of European Center of Disease Control and Prevention.
Methods: All faeces samples of patients with diarrhoea submitted by general practitioners were investigated for CDAD by an enzyme-immunoassay for *C. difficile* toxin A/B. Positive samples were cultured for the presence of *C. difficile*. Isolates were typed by PCR ribotyping. Standardised questionnaires were used to obtain clinical data and patient information using ICD-10 classification.
Results: Of 703 faeces samples submitted in a 6 months period in 2006, 34 (4.8%) contained *Salmonella enteritica* and 21 (3%) *Campylobacter*. *Giardia lamblia* and *Yersinia enterocolitica* were isolated in 2 and 1 patients, respectively. *C. difficile* was detected in 66 (9.3%) of all faeces samples; 13 (26%) patients had moderate–severe diarrhoea and 53 (74%) suffered from mild diarrhoea. Of 66 patients with community-onset CDAD, 31 (47%) were healthcare-associated (onset of symptoms within 48 h following admission or within 4 weeks after discharge) and 53% were community-associated. The mean age of 66 patients (24 males and 41 females) with CDAD was 66 years. The most frequent underlying diseases were cardiovascular disease (41%), urogenital disease (20%), lung disease (14%), diabetes mellitus (11%) and malignancy (9%). Recent antibiotic use was reported by 34 (52%) patients with cephalosporins (33%) and fluoroquinolones (33%) as most frequently. Of 66 patients, 19 (29%) received a PPI and 17 (26%) NSAID. Preliminary results of typing of 14 strains revealed 8 different types with type 001 (21%) and type 015 (14%) as the predominant types.
Conclusion: *Clostridium difficile* was the most frequent cause of diarrhoea in a population of patients who visited the GP because of diarrhoea, but 47% was healthcare-associated.

O330 Predicting *Clostridium difficile* toxin in hospitalised patients with antibiotic-associated diarrhoea

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Objectives: *Clostridium difficile* infection is implicated in 20–30% of cases of antibiotic-associated diarrhoea. Few studies have evaluated risk factors for the development of *C. difficile*-associated diarrhoea (CDAD) in patients with antibiotic-associated diarrhoea. Our objective was to compare clinical characteristics of hospitalised patients who received antibiotics and developed CDAD with hospitalised patients who received antibiotics and developed diarrhoea with negative *C. difficile* toxin assay in their stool.
Methods: A prospective cohort study of hospitalised patients who had received antibiotics and developed diarrhoea were included. CDAD patients were defined as patients who had diarrhoea and was associated with a positive stool toxin A/B enzyme immunoassay test. Univariate and stepwise logistic regression analyses were used for prediction of *C. difficile* toxin. The predictive capability of the model was demonstrated by a receiver operator characteristic (ROC) curve.
Results: Fifty-two (24%) out of 217 patients were found to be positive for *C. difficile* toxin A/B in their stool. The logistic regression model included impaired functional capacity, watery diarrhoea, use of a proton pump inhibitor, use of a histamine receptor blocker, leukocytosis, and hypoalbuminaemia. The area under the receiver operator characteristic curve for the model as predictor to positive stool toxin assay was 0.896 [95% CI: 0.661–1.00; $p < 0.001$], with a 95% specificity and 68% sensitivity.
Conclusions: Our results may help clinicians predict the risk of CDAD in hospitalised patients with antibiotic-associated diarrhoea, guide careful antibiotic prescription and early attention to infection control issues.

O331 Metronidazole and vancomycin outcomes for *Clostridium difficile*-associated diarrhoea in a US hospital database

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Objective: *Clostridium difficile*-associated diarrhoea (CDAD) treatment guidelines recommend metronidazole (MET) first-line and vancomycin (VANC) second, but few studies compare outcomes. The objective of this study is to compare hospital outcomes for identified CDAD patients treated first with MET versus VANC therapy.
Methods: CDAD cases (ICD-9 008.45) January 2004 to June 2005 were identified in a national US hospital database (Premier Perspective). Inpatient cases receiving oral MET or VANC were analysed, and initial dual therapy cases were excluded. Groups were compared on demographics, prior CDAD admissions, and comorbidity proxy measures, the APR-DRG Severity of Illness (SOI) and Risk of Mortality (ROM). Length of stay (LOS), in-hospital mortality, ICU stay, colon resection defined case outcomes. Chi-square and t-tests produced p-values.
Results: We identified 32,325 CDAD cases treated first with MET (89%) or VANC (11%). Cases were similar in age (mean 70 years, $p = 0.38$). Fewer ($p < 0.0001$) MET than VANC cases were female (58% vs 64%), had a principal CDAD diagnosis (19% vs 31%), prior CDAD admission (10% vs 31%) or prior acid suppressive therapy (42% vs 53%). Higher SOI and ROM were observed in MET vs VANC ($p < 0.0001$), with 30% MET and 24% VANC rated extreme SOI. During hospitalisation, 15% VANC cases subsequently received MET, and 12% MET received VANC; 9% MET and 11% VANC received IV MET (unknown if specifically for CDAD), and 1.5% MET and 3.1% VANC received rifampin. MET vs VANC had increased LOS (12.8 vs 11.5 days, $p < 0.0001$), in-hospital death (7.9% vs 6.8%, $p < 0.02$), and ICU stay (23.2 vs 17.7 days, $p < 0.0001$). There was no difference in colectomy (1.0% MET vs 0.8% VANC, $p = 0.37$). Despite lower costs of MET vs VANC therapy, total pharmacy costs were similar ($2,439 vs $2,492, $p = 0.52$), while total hospitalisation costs were higher ($16,953 vs $14,718, $p < 0.0001$).
Conclusion: Most CDAD patients received MET, and modification to initial therapy occurred in similar proportions of MET and VANC cases. Compared to initial VANC therapy, those treated with MET had higher rates of poor discharge outcomes and greater resource use, however comparisons do not adjust for acute comorbidities.

O332 Successful combat against *Clostridium difficile* PCR-ribotype 027 at a regional outbreak from 2003–2006 in the Netherlands

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Background: Since 2003 severe cases of *Clostridium difficile* (CD) associated diarrhoea (CDAD) have been reported in hospitals in North America and Europe caused by a hypervirulent strain CD ribotype 027, toxinotype III. This ribotype is also detected in The Netherlands.

Objective: A descriptive study to the incidence of CDAD after the introduction of specific infection control measures in a region with outbreaks due to type 027.
Methods: A case of CDAD was defined as a patient with diarrhoea and a positive toxin test on a faeces sample (ICTAB, Meridian). Strains isolated from positive faeces samples were further investigated by PCR ribotyping.
Results: The Public Health Laboratory Haarlem, The Netherlands, services an area with 440,000 inhabitants including nursing homes and three hospitals (A, B and C). Monthly admission rates of the latter are 1,050, 1,300 and 1,600, respectively; overall admission rate 47,000 yearly. Retrospective evaluation of laboratory data showed short episodes of CDAD outbreaks in hospital A in October 2002 (incidence 62/10,000 admissions) and June 2003 (121/10,000); in hospital C in December 2003 (50/10,000) and April 2004 (44/10,000). The highest CDAD increase was found in hospital B in August 2004 (>130/10,000). The latter episode was found to be the start of a long-term increase of CDAD with incidence peaks of >100/10,000 admissions per month in all hospitals as well as one nursing home to which hospital patients were transferred. In the 3rd quarter of 2005, 67% of 30 CD strains from hospitalised patients belonged to the hypervirulent ribotype 027. Specific measures against CDAD were introduced in all hospitals and nursing homes: strict barrier precautions as private room, glove use, gowns and hand washing, environmental cleaning with bleach and restrictive use of fluoroquinolones. Also a laboratory algorithm was introduced to investigate all faecal samples from hospitalised patients with diarrhoea. Subsequently, during the 4th part of 2005 until the 3rd part of 2006, CDAD incidence decreased to a mean of 21/10,000 (range 30–13/ /10,000) admissions, corresponding with the national incidence. The percentage type 027 strains decreased from 67% to 36% and 8% in the 3rd 05, 1st 06 and 3rd 06 period, respectively.
Conclusion: Specific hygienic measures in combination with restrictions of antibiotic prescriptions and intensified laboratory surveillance are successful to overcome CD 027 outbreaks.

Bacterial phylogeny and complete genome sequences

S335 Identification of bacterial lineages using multi-strain whole genome microarrays

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Whole-genome multi-strain bacterial microarrays can be used to take a rapid snapshot of gene presence or absence in unsequenced bacterial genomes, and allow large bacterial populations to be interogated for genetic markers of phenotypic, clinical or evolutionary behaviour. We have designed and built a seven-strain *Staphylococcus aureus* microarray, and used it to compare *S. aureus* isolates from nasal carriage, community- and hospital-acquired disease. We found the *S. aureus* genome consists of three parts, core, core variable (CV) and mobile genetic elements (MGE).
About 70% of the genes in a *S. aureus* genome are common to all strains and called core. Surprisingly, about 12% of genes are CV, their presence, absence or variabity can be used to classify an isolate into one of about ten human lineages. This classification matches well with multi-locus sequence typing clonal complexes. Many CV genes are regulators of virulence genes, or known or predicted to be expressed on the bacterial surface and to interact with the host during nasal colonisation and infection. Since each lineage carries a unique combination of CV genes scattered throughout the chromosome, there was likely to be a common *S. aureus* ancestor but early evolutionary divergence of lineages, with possible selection in the nose. MGE often carry key resistance and virulence genes, and show substantial variation within and between lineages, indicating frequent horizontal transfer. Interestingly, we have been unable to identify any markers that differ between carriage and typical invasive community isolates (not CA-MRSA), suggesting community-acquired invasive disease is strongly dependent on host factors.
We have also discovered the likely mechanism controlling the independent evolution of lineages. A restriction modification pathway found in all *S. aureus* called Sau1 can block horizontal transfer of DNA between isolates, and the specificity of the system varies according to lineage. Thus DNA from one lineage is recognized as foreign and digested by other lineages, but as self by isolates of the same lineage. Not only does this explain independent lineage evolution, but has important implications for how *S. aureus* continues to evolve, and in particular to acquire MGE leading to increasingly antibiotic resistant and virulent strains.

Infectious disease challenges in international settings

S337 Baseline laboratory facilities on remote sites in Africa: too much/too little?

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The provision of medical care to privately funded, isolated populations in geographically and socio-economically remote areas of the world pose the challenge of providing maximum laboratory support to medical and para-medical personnel in the most cost effective way.
This requires a pragmatic approach based on a knowledge of the clinically most significant diseases in the area, either due to a high and clinically significant incidence or a high risk of significant morbidity and or mortality due to disease, the outcome of which may require or may benefit from reliable, affordable and readily available laboratory diagnostics.
The author provides a perspective on this challenge based on the provision of medical services to multinational companies operating in remote areas of Africa on a limited budget over a ten year period.
In a setting in which a relatively small number of expatriate and national employees are taken care of with limited funding, the healthcare provider needs to be aware of the health risk profile of the region in which the service is rendered, the likely exposure of the population served to the prevalent diseases and the availability of laboratory tests that could or would make a significant difference to clinical disease management if available
The main limiting factor in the provision of laboratory support to a medical service is funding.
Laboratory equipment may vary from a number of simple dry chemistry based kits to sophisticated spectrophotometer and other technology based analysers.
A number of factors impact on this including ease of operation of the provided tests, sensitivity and specificity, quality assurance and sustainability.
The absence of trained laboratory personnel is most often a significant determinant in the decision making process regarding the level of sophistication that can be achieved.
On the African continent the advent of rapid malaria antigen test kits have had a major impact on suspected malaria case management in the absence of trained miscroscopists.
Long standing main stays of side-room diagnostics such as blood and urine test kits capable of detecting a number of metabolic and other diseases prove to be cost effective, regularly useful, affordable and sustainable whilst sophisticated dry chemistry analysis machines find a very limited application on a remote site with limited medical and other infra-structure.
Dry chemistry tests that may be useful in this setting are superfluous in a setting in which a patient who requires, e.g., regular kidney function monitoring needs to be evacuated due to the overall limitations beset by the area.

S338 Medical challenges in migrants

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Migrants and other mobile individuals, such as migrant workers and asylum seekers, are an expanding global population of growing

social, demographic and political importance. Often, grave disparities exist between place of origin and destination, in particular with relation to health determinants. The effects of those disparities can be observed at both individual and population levels. Migration influences the epidemiology of many diseases globally and in nations receiving migrants. Specific disease-based outcomes may vary between migrant group and location. Traditionally, migration health activities have been designed for national application and lack an integrated international perspective. Present and future health challenges related to migration may be more effectively addressed through collaborative global undertakings. The epidemiological relationships resulting from health disparities in migration are reviewed and the growing role of migration and population mobility in global disease epidemiology is highlighted. Implications for national and international health policy and programme planning are presented.

S340 Emerging viral diseases: fighting an uphill battle

S.K. Lam (Kuala Lumpur, MY)

In the last decade, Southeast Asia has become the epicentre of a number of new disease outbreaks with the potential for global spread. Nipah virus encephalitis first occurred in Malaysia in 1998 and there is serological evidence that the virus has spread to Cambodia, Thailand, China and India. In Bangladesh, Nipah virus or one closely related to it has caused several outbreaks in Bangladesh with a high mortality rate. The outbreak of severe acute respiratory syndrome (SARS) followed by avian influenza H5N1 in China and their subsequent spread led to fear of global pandemics. Many factors accounting for the emergence of new infections must be in place prior to such outbreaks. The convergence of a number of these factors in Southeast Asia makes this region a hotspot for their emergence.

In order to mount a programme to fight emerging viral diseases, it is important to have adequate funds and political commitments. The key to a comprehensive programme includes good surveillance, clinical hospital management, preventing disease spread, strong laboratory support, research and evaluation, and a well-tested national preparedness plan. Surveillance is complicated in the case of a zoonotic disease such as avian influenza H5N1. Hospitals are ill-equipped to cater to large numbers of severely ill patients as in the Nipah virus outbreak in Malaysia. Preventing disease spread in an era of globalisation poses tremendous challenges in developing countries that depend on international trade and tourism. Research and evaluation are greatly hampered by lack of manpower and infrastructure, including the lack of high security laboratories. Although there are guidelines available for pandemic preparedness as in the case of avian influenza, national plans are totally inadequate and do not measure up to expectation.

Despite these inadequacies, developing countries in Southeast Asia are making contingency plans in the event of unexpected viral emergence. There is no doubt that this will be an uphill battle but certain measures initiated in the region will hopefully reduce the spread of emerging viral diseases.

Antimicrobial drug discovery: the road is long ... (Symposium arranged with the ICAAC Program Committee)

S345 What's in the pipeline? Cell wall inhibitors/ miscellaneous metabolism inhibitors

K. Bush (Raritan, US)

Cell wall inhibitors have traditionally provided the infectious disease community with safe and efficacious agents to treat serious bacterial infections. In addition, metabolism inhibitors, singly or in combination, have continued to provide coverage for some of the more resistant pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA).

Of the cell wall inhibitors in the pipeline, β-lactams have been a major component of antibiotic discovery programmes, but lagged in development as multidrug-resistant Gram-positive bacteria, such as MRSA, and cephalosporin-resistant Gram-negative organisms emerged as prevalent nosocomial pathogens. Compounds in late stage development include anti-MRSA β-lactams such as ceftobiprole, the broad-spectrum cephalosporin that has successfully completed two Phase III trials in complicated skin and skin structure infections (cSSSI). Following are two investigational anti-MRSA β-lactams, the anti-MRSA cephalosporin, ceftaroline, that has completed a Phase II cSSSI trial and the carbapenem CS-023/RO-4908463 with in vitro activity against MRSA and cephalosporin-resistant Enterobacteriaceae. Also in development are two additional carbapenems, ME1036, in Phase I studies, with activity against MRSA as well as against most Enterobacteriaceae, and SMP-601/PZ-601 that has demonstrated in vitro activity against many multidrug-resistant Gram-positive and Gram-negative bacteria in preclinical studies.

Other β-lactam agents in development include doripenem, a carbapenem with enhanced activity against *Pseudomonas aeruginosa*; doripenem has recently completed clinical trials for complicated urinary tract infections and intra-abdominal infections. FR264205 is an unusual preclinical cephalosporin with promising in vitro activity against *Helicobacter pylori* and AmpC-hyperproducing *P. aeruginosa*.

Additional cell wall active agents include lipoglycopeptide inhibitors that are active against most Gram-positive bacteria. Telavancin and dalbavancin are in late-stage regulatory reviews, and are characterised by long half lives in humans, with a proposed once-weekly dosing regimen for dalbavancin.

Iclaprim, a dihydrofolate reductase inhibitor that is the most advanced metabolism inhibitor with completion of a Phase III cSSSI clinical trial, demonstrates in vitro antibacterial activity against MRSA, penicillin-resistant pneumococci and other respiratory pathogens. Other metabolism inhibitors that have been described in early development include peptide deformylase inhibitors and inhibitors of fatty acid biosynthesis. However, none of these early metabolism inhibitors have proceeded to proof-of-concept therapeutic studies at this time.

S346 The compound bank approach: complementing to increase the chance of success

T. Trust (Waltham, US)

Since the publication of the first complete bacterial genome in 1995, the genome-derived target based approach to discover new classes of antibacterial drugs with novel mechanisms of action has been employed widely. The literature shows that the success rate of both biochemical screening of individual antibacterial targets, and whole-cell antibacterial screening has been disappointing. In retrospect, this is not surprising given the historical content of the chemical collections that companies employed in their early high throughput screening campaigns. Most companies have engaged in "cleaning-up" their compound libraries in recent years and have increased the molecular diversity of their collection in efforts to increase the chance of success, regardless of therapeutic area. Efforts have also been made to create focussed chemical libraries that are better suited to finding antibacterial compounds. By complementing the screening of diverse, drug-like, infection-friendly compound collections against drugable targets, and early application of structure-based approaches, we should see breakthroughs in the discovery of antibacterial drugs with novel mechanisms of action.

S347 The natural product approach: advances over the last 10 years that make it an attractive approach again

S. Pelzer (Berlin, DE)

There is an urgent medical need to discover and develop new antibiotics, since resistance to antibiotics is becoming an increasingly frequent problem and current development pipelines are lacking innovative, novel antibiotic classes. In the past natural products dominated the field

of antibiotics and have had a significant role in the discovery and development of commercially successful drugs of all classes. Over 75% of the antibacterial new chemical entities (NCEs) introduced worldwide between 1981 and 2002 were based on natural products. The unmatched chemical diversity and complexity of natural products is one reason for their success over those obtained by pure chemical synthesis. Another reason for their superiority can be explained by the fact that their synthesis evolved naturally in response to needs and challenges of the natural environment generating compounds which are pre-selected for activity. Despite these facts, natural product research has recently gone through a phase of reduced interest. Big pharmaceutical companies in particular have downgraded or even stopped this kind of research. The reasons for this development may be that natural products are often produced in low quantities and as mixtures of similar compounds, the rediscovery of known compounds and the challenge of natural product derivatisation using classical chemical means.
The past few years have witnessed major developments in the use of innovative natural product related technologies, such as fermentation optimisation, separation, structure elucidation and dereplication allowing much faster access to sufficient quantities of pure natural compounds. The application of modern medicinal chemistry adapted to the special needs of natural products is also an efficient way to revisit and recycle old antibiotic classes. The use of modern Genome-based technologies, established in the past few years, offers the opportunity to increase the attractiveness of natural products. Genome-based screening technologies provide fast access to the enormous genetic potential of Actinomycetes, soil bacteria known to represent one of the most important sources for bioactive metabolites. Additionally, genetic engineering technologies will help to overcome two of the main hurdles connected to natural products, the difficulty in derivatising complex structures and the quantitative improvement of the production yield.
By focussing on examples in the field of genome based screening and genetic engineering this presentation will give an overview of major improvements that may lead to a rediscovery of natural product based drugs to meet the urgent need for new antibiotics.

Respiratory pathogens and vaccination

O348 Clinical evaluation of a new ID-Tag RVP assay for the detection of 20 respiratory viruses

J. Mahony, S. Chong (Hamilton, CA)

Objectives: To evaluate the performance of the ID-Tag RVP test for the detection of respiratory viruses.
Methods: NP specimens [N = 227] were collected from symptomatic patients under ERB approval and were tested by the ID-Tag RVP test (TmBioscience Corp'n, Toronto, Canada) and conventional DFA plus culture. The RVP test is a new test for the detection of 20 respiratory viruses that uses multiplex PCR, a Universal Array of oligonucleotides (TmBioscience), and a fluidic microbead array (Luminex X-Map). The test detects Influenza A (subtypes H1, H3, and H5 Asian lineage), Influenza B, Parainfluenza types 1, 2, 3, 4, RSV types A and B, Adenovirus, Metapneumovirus, Rhinovirus/Enterovirus, Coronavirus 229E, OC43, NL63, HKU1, and (SARS-CoV). The ID-Tag RVP test was performed according to manufacturer's instructions. Briefly, viral nucleic acid was amplified by a multiplex PCR followed a multiplex Target Specific Primer Extension (TSPE) reaction and sorting of TSPE products using the Luminex X-Map system. DFA and culture were performed using MCabs and R-Mix shell vials (Diagnostic Hybrids Inc.). For discordants and RVP positives where the targets were not tested by DFA/culture, a second PCR (unique primers) and sequencing was performed as the comparator test.
Results: Twenty-two of 227 specimens (9.7%) failed to give a signal for the internal control indicating extraction failure or was called equivocal for at least one target. Of the 206 specimens analysed, 135 were RVP+ DFA/culture+ and 37 were RVP– DFA/culture– concordant. There were 7 RVP– DFA/culture+ and 27 RVP+ DFA/culture– discordants. After resolution of discordants RVP had a sensitivity of 95.8% (138/144) compared with 93.7% (135/144) for DFA/culture. RVP detected an additional 26 confirmed positives including 22 Rhino/Entero, 1 NL63, and 3 HKU1 that are not routinely tested by DFA or culture and 4 additional Flu B positives that were missed by DFA/culture.
Conclusion: The ID-Tag RVP test is more sensitive than DFA/culture and detects a number of respiratory viruses not routinely tested for. Overall 22.5% additional positive specimens were detected that were either missed by DFA/culture or not tested for. The ID-Tag RVP test should improve the ability of hospital and public health laboratories to diagnose viral RTIs in a hospital or community outbreak situation.

O349 Molecular epidemiology, genotypes and recombination of coronavirus HKU1, a novel human coronavirus associated with respiratory tract infections

P.C.Y. Woo, S.K.P. Lau, C.C.Y. Yip, Y. Huang, H.W. Tsoi, R.W.S. Poon, B.H.L. Wong, K.H. Chan, K.Y. Yuen (Hong Kong, HK)

Objectives: Recently, we described the discovery of a novel group 2 coronavirus, coronavirus HKU1 (CoV-HKU1), from a patient with pneumonia. In this study, we examined the molecular epidemiology, genotype distribution and recombination in CoV-HKU1.
Methods: We collected nasopharyngeal aspirates of patients with respiratory tract infections over a two-year period. The complete genomes of a total of 22 strains of CoV-HKU1 were sequenced and compared. Genotypes and possible sites of recombination were determined.
Results: Phylogenetic analysis of 24 putative proteins and polypeptides showed that the 22 CoV-HKU1 strains fell into three clusters (genotype A, 13 strains; genotype B, three strains and genotype C, six strains). However, different phylogenetic relationships among the three clusters were observed in different regions of their genomes. From nsp4 to nsp6, the genotype A strains were clustered with the genotype B strains. For nsp7 and nsp8, and from nsp10 to nsp16, the genotype A strains were clustered with the genotype C strains. From hemagglutinin esterase (HE) to nucleocapsid (N), the genotype B strains were clustered closely with the genotype C strains. Bootscan analysis showed possible recombination between genotypes B and C from nucleotide positions 11500 to 13000, corresponding to the nsp6/nsp7 junction, giving rise to genotype A; and between genotypes A and B from nucleotide positions 21500 to 22500, corresponding to the nsp16/HE junction, giving rise to genotype C. Multiple alignments further narrowed the sites of cross-over to a 143-bp region between nucleotide positions 11750 and 11892, and a 29-bp region between nucleotide positions 21502 and 21530. Genome analysis also revealed variable numbers of tandem copies of a perfect 30-base acidic tandem repeat which encodes NDDEDVVTGD and variable numbers and sequences of imperfect repeats in the N-terminal of nsp3 inside the acidic domain upstream of papain-like protease 1 among the 22 genomes. All 10 CoV-HKU1 with incomplete imperfect repeats (1.4 and 4.4) belonged to genotype A.
Conclusions: Three genotypes, genotype A, genotype B and genotype C, exist in CoV-HKU1. Analysis of a single gene is not sufficient for genotyping of CoV-HKU1, but would require amplification and sequencing of at least two gene loci, one from nsp10 to nsp16 (e.g. pol or helicase) and another from HE to N (e.g. spike or N).

O350 Epidemiology and diagnosis of epidemic and avian influenza in Australia

C. Baleriola, S. Stelzer, R. Escott, P. Kirkland, P. Robertson, W. Rawlinson (Randwick, North Ryde, Candem, AU)

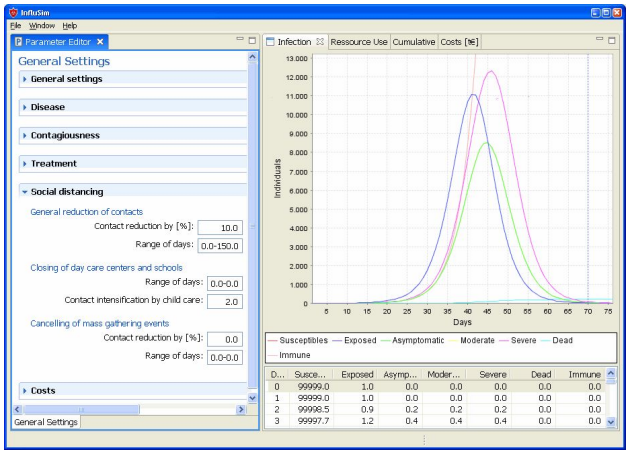
Influenza activity in temperate regions of Australia typically occurs between May and September, whereas in tropical regions influenza can occur any time throughout the year.
The main purpose of this presentation is to outline the epidemiology of human influenza during 2001–2006 in NSW. The second purpose is to report a quality assurance programme for laboratory diagnosis of influenza throughout Australia, including avian influenza.

Epidemiological data from NSW, Prince of Wales Hospital (Sydney) shows that Respiratory syncytial virus (RSV) is the first respiratory virus emerging in the winter season. Influenza A infections typically last for 4–5 weeks during August and September. Infections with other respiratory viruses such as Parainfluenza virus 3 and human metapneumovirus occur from June to late November and December. Summary data from the last 5 years will be presented.
Influenza data from 2001 to 2006 indicate that the first ones affected early in the season are children and young adults, whereas during peak season the majority are between 20 and 50 years of age. There is an even distribution between the number of influenza attacks among males and females (51% and 49% respectively). From a total of 500 specimens tested for respiratory viruses during 2001–2006, 51% were true influenza cases, 10% RSV and 17% rhinovirus. Data collected from the Prince of Wales Hospital is submitted on a weekly basis to the New South Wales Department of Health as part of the NSW Influenza Surveillance Program.
During 2006 we have undertaken a Quality Assurance Program aiming to improve detection and diagnosis of HPAI H5N1 among Australian and Asian Laboratories in collaboration with the Royal College of Pathologists Australia (RCPA) and the Elizabeth MacArthur Agricultural Institute, integrating human and veterinary health laboratories. The project assesses H5 diagnosis accuracy using serological and molecular techniques, utilising a new avian influenza module within the Serology Quality Assurance Program (SQAP). The report from the first Nucleic acid testing panel is already available to the 30 participant laboratories. The results suggest that testing laboratories around Australia and Asia generally utilise accurate and sensitive methods for detection of H5N1, although some laboratories are only testing for Influenza A and are currently not performing specific H5N1 tests.

O351 Model-based pandemic influenza preparedness planning

M. Schwehm, H. Duerr, S. Brockmann, M. Eichner (Tübingen, Stuttgart, DE)

Objectives: Influenza pandemic preparedness plans are currently being developed on national and international levels. Planning public health responses rely on predictive models by which the impact of different intervention strategies can be evaluated. Previous research has rather focused on producing predictions for certain localities or under specific conditions and on the administration of antiviral drugs. The effectiveness of these interventions depends on various factors which must be explored by sensitivity analyses, based on mathematical models. We investigate how pharmaceutical and non-pharmaceutical interventions can mitigate an influenza pandemic and examine how intervention schedules, restricted stockpiles and contact reduction (social distancing measures and partial isolation of cases) determine the course of a pandemic wave and the success of interventions.
Methods: We provide the freely available planning tool InfluSim (figure), a deterministic compartment model, which is based on a system of over 1,000 differential equations and designed to operate with an optimal combination of the competing requirements of precision, realism and generality. It allows for producing time courses and cumulative numbers of influenza cases, outpatient visits, applied antiviral treatment doses, hospitalisations, deaths and work days lost due to sickness, all of which may be associated with economic aspects.
Results: The model shows that a timely application of antiviral drugs combined with a quick implementation of contact reduction measures is required to substantially protract the peak of an influenza epidemic and reduce its height. Delays in the initiation of antiviral treatment (e.g. because of parsimonious use of a limited stockpile) result in much more pessimistic outcomes and can even lead to the paradoxical effect that the stockpile is depleted earlier compared to early distribution of antiviral drugs.
Conclusion: InfluSim efficiently assists public health planners in designing optimal interventions against pandemic influenza. It reproduces the infection dynamics of pandemic influenza like complex computer simulations while offering at the same time reproducibility, higher computational performance and better operability. When controlling pandemic influenza, pharmaceutical and non-pharmaceutical measures should not be used exclusively. Contact reduction measures must be part of mitigation strategies and have the advantage to be not limited per se.



O352 Evaluation of the antibody responses induced by the 2006–2007 influenza vaccine

Y.-J. Chan, S.-J. Hwang, J.-C. Lin, C.-H. Tsai, C.-Y. Liu, S.-D. Lee, D.M. Ho, C.-H. Lee (Taipei, TW)

Objectives: Although influenza (Flu) is an old infectious disease, it will cause pandemics and requires global cooperation for control and prevention. In addition to the avoidance of exposure during the influenza season vaccination has been the most effective modality for preventing influenza epidemics. However, due to the genetic variability of the influenza it requires vaccination every year. Our Department of Health supported free influenza vaccination for aged people (>65 year-old) for years and the host antibody responses are evaluated.
Methods: A total of 89 paired sera was collected before and 3 weeks after the vaccination with one dose of a commercial trivalent influenza vaccine for 2006–2007. The paired samples were tested for influenza A and B antibodies by complement fixation (CF) and quantitative enzyme-linked immunosorbent assay (ELISA). In addition, 83 paired sera were tested by haemagglutination inhibition (HI) using an A/New Caledonia/20/99-like strain. There were 49 males and 34 females, and 47 people were older than 65.
Results: Our results indicated that the CF response rates (defined as 4-fold titer increase of the paired sera) for both influenza A and B were low (33.7% and 15.7%, respectively). However, the pre-immunised ELISA titers for both influenza A and B were much higher than normal (average 98.3±45.6 RU/mL for Flu A and 176±57.5 RU/mL for Flu B; normal <22 RU/mL). The overall response rate for HI was 32.5% (27/83), but the response rate of the aged (>65 year-old) was lower (27.7%, 13/47) than people <65 year-old (38.9%, 14/36). However, the rate of pre-immunised sera with protective HI titer (HI $\geqslant$ 40) was 80.7% (67/83).
Conclusion: The antibody responses induced by the 2006–2007 influenza vaccine were low, but the pre-immunised antibody positive rates and titers were high. The phenomenon might reflect the quality of the vaccine and the consequences of previous vaccination.

O353 Perception of adverse-effect risks and receipt of influenza vaccination among hospital personnel

B.P. Ehrenstein, F. Hanses, S. Blaas, F. Mandraka, F. Audebert, B. Salzberger (Regensburg, DE)

Objectives: Hospital personnel (HP) are at risk for contracting influenza and may transmit influenza to patients. Although, influenza vaccination of HP significantly reduces the overall mortality of patients, vaccination

rates in many hospitals remain low. Surveys among HP delineated fear of adverse effects (AE) as an important reason for HP not to get vaccinated. We surveyed HP to delineate their appraisal of influenza vaccine AE rates and to correlate correct knowledge of AE rates with the receipt of influenza vaccination.
Methods: In February 2006, we distributed anonymous self-administered, multiple-choice paper questionnaires to the 637 physicians and final-year medical students (FYMS), 994 nurses, and 267 hospital administrators at a university tertiary care hospital. Questions pertained to the incidence and complication rates of influenza, to the general knowledge about, and the indications for influenza vaccination, and to the rates of twelve possible influenza-vaccination AE. We also retrieved demographic data, the receipt of influenza vaccination in the current season (2005/2006), and the most important reason to get or not get vaccinated.
Results: 647/1898 (34%) surveys were returned. 345/647 (53%) respondents had received influenza vaccination in 2005/2006. 127/302 (42%) 2005/06 non-vaccinated HP stated that vaccine AE were the most important reason not to get vaccinated. Overall, respondents underestimated the incidence rates of non-severe AE (headache, muscle aches, fever, chills, painful injection site, and >2 days absenteeism) and overestimated severe AE rates (skin necrosis, severe hepatitis, acute renal failure, encephalitis, Guillain-Barré syndrome, permanent neurological damage). In a multivariate analysis among physicians and FYMS, receipt of influenza vaccination 2005/06 was independently associated with above-average knowledge about influenza vaccine (OR 2.4) and correct appraisal of severe AE rates (OR 3.7), among nurses with above-average knowledge about the influenza vaccine (OR 2.1) and an underestimation of non-severe AE rates (OR 3.6).
Conclusion: HP overestimated the severe AE rates and underestimated the non-severe AE rates of influenza vaccination. The appraisal of these AE rates appears to be very important in the decision of HP to get vaccinated. A better mediation of the actual very low rates of severe adverse effects may improve the influenza-vaccination rates among HP.

O354 Cross-subtype immunity against avian influenza in humans recently vaccinated for the influenza season

C. Castilletti, C. Gioia, L. Bordi, C. Agrati, M. Tempestilli, R. Chiappini, P. Piacentini, S. Squarcione, G. Ippolito, M. Capobianchi, F. Poccia (Rome, IT)

Objectives: Avian H5N1 influenza viruses could be transmitted to humans, resulting in severe or fatal disease. Aim of this study was to evaluate the immune cross-reactivity between human and avian (H5N1) influenza strains in healthy donors vaccinated for seasonal H1N1/H3N2 influenza A.
Methods: Healthcare workers wishing to receive seasonal influenza vaccination at the Spallanzani Institute were enrolled. Blood samples for the assessment of humoral and cell-mediated responses were obtained before and 30 days after vaccination. The frequency of circulating antigen-specific CD4 and/or CD8 T-cells in healthy donors enrolled in the study were analysed by flow cytometry, using intracellular cytokine staining assay after the expansion of effector-cells in vitro. Human sera from the same donors were tested for their HA-inhibition activity against vaccine preparation and neutralisation activity against H5N1 virus.
Results: Our data indicate that vaccination may boost cross-subtype cellular and/or humoral immunity against H5N1 influenza. Specifically, H5N1-specific CD4 T-cell frequency was significantly higher in pluri-vaccinated versus first flu-vaccinated donors. The main target for cross-reactivity between H5N1 and H3N2/H1N1 strains was N1. No correlation between influenza-specific CD4 T-cells and humoral response was observed, suggesting that this response was mainly CD4 T-cell-independent. Differently, CD4 T-cells may help for anti-influenza CD8 T-cell response. Furthermore, human sera from the same donors, tested for their HA-inhibition activity against vaccine preparation, showed a significant rise (73.7%) after vaccination. The same sera were tested for their H5N1 neutralisation activity and 34.2% of subjects was able to show anti-H5N1 antibody rise after seasonal influenza vaccination, showing the existence of an antibody-dependent cross-type immunity. No correlation between influenza specific CD4 T-cells and humoral responses were observed, suggesting that this type of antibody response was mainly CD4 T-cell independent.
Conclusion: In this study, we demonstrated that vaccination against seasonal influenza might induce both cellular and humoral cross-reactive immunity against H5N1 avian influenza. This cross-type immunity may represent an important component of the immune response against novel influenza A infections.

O355 Potential impact of pneumococcal vaccination in Denmark

Z.B. Harboe, P. Valentiner-Branth, T. Hjuler, J.J. Christensen, L. Lambertsen, K. Mølbak, H.B. Konradsen (Copenhagen, DK)

Background: Since 2000, the pneumococcal 7-valent protein-conjugate vaccine (PCV-7) has been implemented in the childhood vaccination programme in several countries. In Denmark (DK), the PCV-7 is only recommended for children in high-risk groups.
Objective: To describe incidence and antibiotic resistance of invasive pneumococcal disease (IPD) and estimate the direct and indirect effect of PCV introduction in DK.
Methods: Review of demographic and laboratory data from IPD cases between 2000 and 2005. Data on pneumococcal meningitis were obtained from the Notifiable Infectious Disease Register. Considering the local serotype distribution, the direct effect of PCV-7, -10, and -13 was estimated in children under 5 years (<5 y), assuming a 90% vaccination coverage and a 97% effectiveness in reducing IPD caused by vaccine-serotypes. Herd immunity effect was estimated assuming a 40% reduction of IPD caused by vaccine-serotypes.
Results: (1) Incidence: The total incidence remained stable throughout the period, ~20/100,000 population, whereas the incidence in <5 y increased from 25 to 34. The highest incidence was observed among adults >65 years, ~69 per 100,000. 92% of cases presented as bacteraemia. Approx. 100 cases of pneumococcal meningitis were registered annually, with an overall mortality of 20%. (2) Vaccine effectiveness: The overall serotype coverage among IPD < 5 y was estimated as: 64% (PCV-7), 82% (PCV-10), and 91% (PCV-13). With an annual average of 86 cases of IPD < 5 y the number of vaccine-preventable cases would be: 48 (PCV-7), 61 (PCV-10) and 68 (PCV-13). The annual number of IPD > 5 y protected by herd immunity would be: 144 (PCV-7), 237 (PCV-10) and 277 (PCV-13). In terms of mortality, 2.1 children <5 y die annually. Introduction of PCV would prevent 1.2 deaths (PCV-7), 1.5 (PCV-10) and 1.7 (PCV-13). The annual number of deaths among IPD > 5 y is 199; 30 fatal cases would be prevented by PCV-7, 46 by PCV-10 and 55 by PCV-13. (3) Antibiotic resistance: 2.5% of the isolates tested for penicillin susceptibility had MIC > 0.125 ug/mL, none had MIC > 2 ug/mL. 5% of isolates presented erythromycin MIC > 1.5 ug/mL.
Conclusions: The incidence of IPD < 5 y increased during 2000–2005 in DK. The PCV-10 and -13 would provide higher protection than PCV-7 based on the serotype distribution, mainly due to presence of serotype 1. Monitoring serotypes, resistance, and mortality related to IPD is essential in order to evaluate the effect of vaccine introduction.

O356 Comparison of immunogenecity of pneumococcal polysaccharide vaccine with protein conjugate vaccine in Korean adults

S.Y. Lim, H.J. Lee, S.Y. Woo, J.Y. Seoh, K-H. Kim (Seoul, KR)

Objective: To compare the immunogenicity of a 7-valent pneumococcal conjugate vaccine (PCV) to a 23-valent polysaccharide pneumococcal vaccine (PPV) in Korean adults.
Methods: Twenty-seven and 20 healthy adults were immunised with either a PCV or a PPV intramuscularly. Sera were obtained before and at 4 weeks after immunisation to determine IgG pneumococcal antibody titers by enzyme-linked immunosorbent assay and opsonisation tier by opsonophagocytic killing assay to the 7 serotypes represented in the PCV.
Results: Antibody titers and opsonisation titers to 7 pneumococcal serotypes were significantly increased after immunisation in PCV and

PPV groups. Post-immune antibody titers to serotype 4, 18C and 23F were significantly higher in PCV groups than in those in PPV group. Also PCV elicited significantly higher fold rise of opsonisation titer compared with PPV in serotype 4, 18C and 23F. Antibody titers and opsonisation titers to 7 serotypes have good correlation in pre-immune sera as well as post-immuue sera in both groups. More than 95% of subjects in both groups have antibody titer ⩾1 μg/mL to 7 serotypes after immunisation.

Serotype	Vaccine	EIA (μg/mL)			OPKA (opsonisation titer)		
		Pre-im	Post-im	Fold incr	Pre-im	Post-im	Fold incr
Pn 4	PPV	0.43	2.21*	4.83	17	1275*	73
	PCV	0.48	6.56*#	13.60#	8	1656*	212#
Pn 6B	PPV	1.06	6.17*	5.36	350	5958*	17
	PCV	2.88	13.34*	4.63	73	3766*	52
Pn 9V	PPV	1.08	6.05*	5.27	464	4424*	10
	PCV	1.14	7.91*	6.94	59	3686*	63
Pn 14	PPV	3.02	18.70*	4.91	237	4846*	20
	PCV	2.82	26.76*	9.49	48	2311*	48
Pn 18C	PPV	1.1	5.80*	4.58	636	5583*	9
	PCV	1.4	12.99*#	11.29#	12	2199*	184#
Pn 19F	PPV	3.28	8.42*	2.25	40	2035*	51
	PCV	3.66	12.60*	3.44	31	1142*	37
Pn 23F	PPV	0.6	3.22*	4.81	478	2840*	6
	PCV	1.15	10.92*#	9.48#	21	1280*	60#

[a]Pre-im, pre-immune; Post-im, post-immune; Fold incr, fold increase.

Conclusions: Quantitative and qualitative antibody responses to PCV and PPV are good in Korean adults. PCV is more immunogenic than PPV to serotype 4, 18C and 23F.

O357 Differential propensity of serotype groups to switch and acquire multidrug-resistance after the introduction of the pneumococcal conjugate vaccine in the USA

R.M. Mera, L.A. Miller, H.A. Madsen, M.A. Pentella, T.R. Fritsche, R.N. Jones (Durham, Upper Providence, Iowa City, North Liberty, US)

Objectives: The *S. pneumoniae* heptavalent conjugate vaccine, introduced in February 2000, covered 73% of the US paediatric population in 2004. Changes over time in serotype prevalence and multidrug-resistance (MR) to antibiotics were evaluated using results from a longitudinal surveillance programme (SENTRY).
Methods: The study included 704 isolates: 301 from years before the introduction of the vaccine (1998–1999) and 298 post-release (2003–2004). Key demographic data, serotype and resistance profiles for 5 antimicrobial classes were analysed. Strains displaying resistance (defined as non-susceptibility based on CLSI breakpoints) to >2 classes were considered MR. Statistical analysis was carried out using linear mixed effects models for repeated measures.
Results: After adjusting for age, invasive status and region, a multivariate logistic regression model showed that non-vaccine (NV) isolates are 1.9 times (1.3–2.7, $p = 0.03$) more likely to be MR in the post-vaccine release period. When serotypes are divided into groups according to the prevalence of multiple resistance and their propensity to expand (i.e. become more prevalent after introduction of the vaccine), we found that the serotype 19 not F was 4.4 times more likely to expand (2.1–9.1) than other NV serotypes ($p < 0.0001$). A group formed by serotypes [11, 15, 33, 35] is also more likely to increase in prevalence, OR 2.4, 1.3–4.5, $p = 0.01$) than other NV serotypes. Details in resistance prevalence and proportions at each point in time are shown in the table.
Conclusion: Groups of both vaccine and non-vaccine serotypes can be categorised according to their propensity to expand and to acquire MR. Serotype 19F slightly decreases in prevalence but increases in MR rate, while serotype 19 not F both increases in prevalence and acquires resistance at a rate that is proportional to the expansion process. About half of the NV serotypes rapidly increased both in prevalence and resistance. Vaccine serotypes (excluding 19F) decreased in prevalence but not in resistance.

Serotypes[a]	Prevalence			Multiple resistance		
	Before	After	*n*	Before	After	*p*
PCV7						
19F	20.7%	17.6%~	135	58.9%	80.6%↑	0.009
14, 6B, 9V	31.0%	8.2%↓	138	56.0%	72.0%~	NS
23F, 18C, 4	16.8%	3.4%↓	71	33.3%	50.7%~	NS
NV						
11, 15, 33, 35	5.4%	19.6%↑	88	0%	20.3%↑	0.032
19 not F	3.1%	20.5%↑	83	27.3%	72.2%↑	0.003
6 not B	8.2%	8.8%~	60	58.1%	55%~	NS
Other NV	14.8%	21.9%↑	129	15.4%	7.8%~	NS

[a]PCV7: vaccine serotypes; NV: non-vaccine serotypes.
↑: Significant increase: ↓: Significant decrease; ~. no significant change.

Resistance in clinical isolates

O358 Increasing resistance to quinolones and expanded-spectrum cephalosporins in commensal *Escherichia coli* from children living in urban areas of Latin America: a report from the ANTRES research project

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Objectives: ANTRES is a research project aimed at describing antimicrobial use and resistance in low-resource countries of Latin America. In this project, dissemination of microbial drug resistance is monitored in commensal *Escherichia coli*, which has largely been exploited as an indicator for similar studies. In the baseline study, carried out in 2002, we observed a high rate of faecal carriage of *E. coli* with acquired resistance to several antimicrobial agents. Here we report the results of a second cross-sectional study conducted in 2005 to evaluate the evolution of antimicrobial resistance rates in the studied areas.
Methods: Rectal swabs were collected from 3,193 healthy children aged 6–72 months, living in 4 urban areas (2 in Bolivia and 2 in Peru). Samples were processed by a rapid screening method which allowed detecting of *E. coli* resistant to ampicillin (AMP), ceftriaxone (CRO), tetracycline (TET), chloramphenicol (CHL), kanamycin (KAN), gentamicin (GEN), amikacin (AMK), streptomycin (STR), trimethoprim-sulphamethoxazole (SXT), nalidixic acid (NAL), and ciprofloxacin (CIP). Sampling strategy and microbiological processes were the same as in the baseline study.
Results: The data from the 2005 survey confirmed the high resistance rates for AMP (96% vs. 95%), SXT (94% vs. 94%), TET (93% vs. 93%) and CHL (69% vs. 70%) observed in the baseline study, and showed a remarkably increase in the resistance rates to quinolones (57% vs. 35% for NAL and 33% vs. 18% for CIP) and CRO (1.7% vs. 0.1%). A significant increasing trend was also observed for STR and GEN (92% vs. 82% and 27% vs. 21%, respectively).
Conclusion: The results of this study confirmed the magnitude of the problem of antimicrobial resistance in these low-resource countries and evidenced an alarming increase in the resistance rates to quinolones and to expanded-spectrum cephalosporins.
ANTRES project, supported by EU INCO-DEV, ICA4-CT-2001–10014

O359 Is the ratio of associated *Escherichia coli* resistance comparable over Europe?

N. van de Sande-Bruinsma, G. Kahlmeter, M. de Kraker, E. Tiemersma, J. Monen, H. Grundmann and EARSS participants

Introduction: The EARSS database discloses increasing levels of antimicrobial resistance in *Escherichia coli* in most parts of Europe. We investigated the levels of associated resistance in the *E. coli* database.
Methods: Invasive *E. coli* susceptibility results to aminopenicillin (AMP), 3rd-gen cephalosporins (3CP), fluoroquinolones (FQ) and aminoglycosides (AG) from 2005 were extracted for 7 countries representing different geographic areas and levels of antimicrobial resistance: the Czech Republic (CZ), Germany (DE), Ireland (IE), Finland (FI), France (FR), Slovenia (SI) and Spain (ES). Associated resistance was defined as "resistance to one drug in the presence of resistance of another drug". The levels of associated resistance were determined. For each drug the levels of resistance was determined for isolates susceptible and resistant to the other drugs.

Table. Minimum (min) and maximum (max) % resistance reported per antimicrobial group, and level of associated resistance within these groups of resistant isolates

Resistant to:	Min/max resistance		Associated resistance versus (resistance in susceptible group)[a]			
	(country)		AMP	3CP	FQ	AG
Aminopenicillins (AMP)	Min	36% (FI)	–	7% (0%)[a]	21% (2%)	7% (0%)
	Max	68% (IE)	–	5% (0%)	24% (2%)	11% (1%)
3rd gen cephalosporins (3CP)	Min	1% (FR)	99% (49%)	–	69% (10%)	42% (4%)
	Max	8% (ES)	100% (59%)	–	70% (25%)	35% (8%)
Fluoroquinolones (FQ)	Min	9% (FI)	86% (31%)	22% (1%)	–	26% (0%)
	Max	28% (ES)	86% (52%)	19% (3%)	–	29% (2%)
Aminoglycosides (AG)	Min	3% (FI)	97% (34%)	66% (1%)	91% (6%)	–
	Max	10% (ES)	93% (58%)	27% (5%)	82% (22%)	–

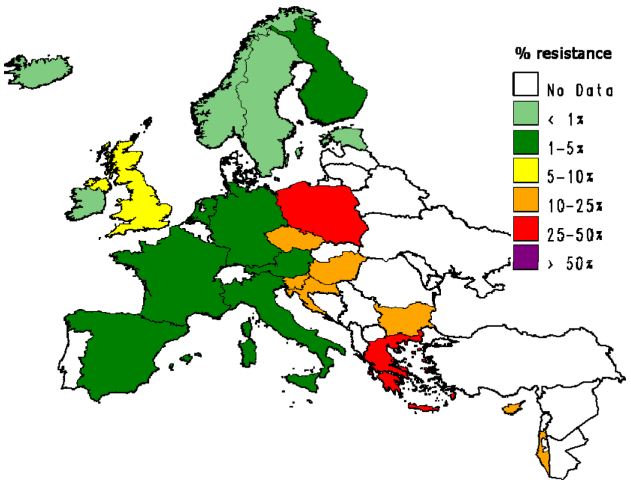
[a]Interpretation: "In Finland 36% of *E. coli* were resistant to AMP; in these resistance to 3CP was 7% as compared to 0% in the 64% not resistant to AMP."

Results: Overall, most if not all resistance to a drug was in the presence of resistance to one or more of the other drugs. More than half of the isolates resistant to 3CP were resistant to FQ. Whereas only 3–10% of all isolates were resistant to AG, similarly almost all isolates resistant to AG were also resistant to FQ (82–91%) as compared to the much lower rates in those sensitive to AG (6–22%). Large differences were observed between countries in antimicrobial resistance rates, whereas the levels of associated resistance among the group of resistant isolates did not vary to the same extent (Table).
Conclusions: (i) The level of associated resistance for the isolates resistant to the different antimicrobial groups under EARSS study seemed comparable between countries, irrespective of their differences in overall resistance proportions. (ii) The group of isolates resistant to one group of antibiotics was more likely to be resistant to other antimicrobial groups (under EARSS surveillance) compared to their susceptible counterparts.
The observation that most resistance is associated with other types of resistance and that the level of associated resistance seems comparable between countries would suggest that the overall reduction of the antibiotic use is probably more important in the society as a whole than targeted reduction of single drugs. Our analysis suggest that efforts should be focused on these 'multi' resistant strains.

O360 Multidrug-resistance among invasive *Klebsiella pneumoniae* in Europe in 2005, the first full year of EARSS reporting

J. Monen, N. van de Sande-Bruinsma, E. Tiemersma, M. de Kraker, H. Grundmann and EARSS participants

Objectives: We explore the resistance to important antibiotic groups of invasive *Klebsiella pneumoniae* isolates reported to the European Antimicrobial Resistance Surveillance System (EARSS) by participating laboratories.
Methods: Participating laboratories carry out routine antimicrobial susceptibility testing (AST) for invasive *K. pneumoniae* isolates. The EARSS protocol includes aminoglycosides, fluoroquinolones and third-generation cephalosporins, but results on carbapenems are also accepted. EARSS accepts the interpretations as defined by the guidelines used by the participating laboratories. Data are collected at the national level and forwarded to EARSS at the National Institute for Public Health and the Environment (RIVM) in Bilthoven, The Netherlands.
Results: In the year 2005, 23 countries reported AST results for 4,929 *K. pneumoniae* isolates. Of these, 4,597 isolates were tested for all classes in the protocol. Six countries reported proportion of less than 1% for the combined resistance against aminoglycosides, fluoroquinolones and third-generation cephalosporins, 7 reported between 1% and 5%, one country between 5% and 10%, 7 between 10% and 25% and 2 more than 25% (see figure). The resistance to carbapenems was <1% in all countries but one, the exception being Greece that reported 25% combined resistance to all 4 groups.



Klebsiella pneumoniae. Combined resistance to aminoglycosides, fluoroquinolones and third-generation cephalosporins.

Conclusion: Our data suggest that multidrug resistance among *K. pneumoniae* is high in many European countries, but that carbapenems can still be used in most of them. The data from Greece predominantly reflect the situation among ICU patients (for which more blood-culture results were available). It must be kept in mind that most guidelines (including CLSI) use breakpoints which are not designed to detect metallo-β-lactamases and that therefore the dissemination of this important resistance trait cannot be deduced from the EARSS data at present.

O361 The prevalence and genetic relatedness of KPC-possessing *Klebsiella pneumoniae* isolates from a United States hospital

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Background: Carbapenem resistance due to KPC-possessing *Klebsiella pneumoniae* is a major healthcare concern. KPC-1 was first identified in South Carolina in 2001 and since then KPC-1 and the KPC-2 and KPC-3 derivations have been identified throughout the USA, Southeast

Asia, and Europe. Following the identification of the first imipenem-resistant, KPC-positive *Klebsiella pneumoniae* isolate in our institution, we initiated a prospective study from August 2006 to November 2006 of 108 consecutive isolates of this organism to determine the prevalence and genetic relatedness of KPC-positive strains.
Methods: KPC-specific polymerase chain reaction was performed on total DNA and plasmid DNA from 108 consecutive *Klebsiella pneumoniae* isolates. Repetitive sequence PCR was performed on all KPC-positive isolates to determine genetic relatedness
Results: Of the 108 isolates collected over the four-month period, 13 (12.0%) were shown to harbour the KPC ORF by PCR. Six of the 13 isolates were identified from three patients, while each of the remaining seven came from individual patients. Eight of the eleven isolates were resistant to imipenem by disk diffusion testing while three of the isolates were fully susceptible. Plasmids containing the KPC gene were isolated and verified by PCR from the 8 imipenem-resistant isolates. In contrast, we were unable to isolate any plasmids from the 3 imipenem-sensitive strains; however, they were positive for the KPC ORF using PCR on total genomic DNA. Two distinct clusters of *Klebsiella pneumoniae* were seen following repetitive sequence PCR. The first cluster of 7 isolates was 86.0% identical suggesting a clonal expansion while the other 5 isolates were unrelated. As expected, multiple isolates from single patients were between 94.8% and 97.4% identical.
Conclusions: The prevalence of KPC-possessing *Klebsiella pneumoniae* in our institution has risen to 9.2% in 4 months thereby compromising empiric treatment options for patients infected with *Klebsiella pneumoniae*. Both a clonal expansion and horizontal plasmid transfer appear to be involved in KPC dissemination. In addition, these data suggest that the KPC ORF may reside in the chromosome and is not expressed until the proper inducing agent is present or that the KPC ORF may reside on a transposable element that is not expressed until transferred into a plasmid.

O362 *Pseudomonas aeruginosa* resistance rates in association with level of hospital care: data from the EARSS

E.W. Tiemersma, N. van de Sande, G. Kahlmeter, M. de Kraker, J. Monen, H. Grundmann and EARSS participants

Objective: *Pseudomonas aeruginosa* (PAE) is known to cause problems mainly in severely immunocompressed patients. This frequently involves multiresistance phenotypes. PAE resistance rates can reach levels of over 10%. Since July 2005, EARSS has been collecting antimicrobial susceptibility testing (AST) data from invasive PAE isolates. Here, we report for the first time the data for 2006 and relate these to hospital characteristics. We investigated the potential importance of levels of hospital care.
Methods: Participating laboratories carry out routine AST for invasive PAE isolates. Data are collected at national level and are forwarded to the EARSS database. AST data and information on hospital characteristics were available for 2,316 isolates from 262 hospitals in 20 countries. AST data included aminopenicillins, ceftazidim, fluoroquinolones, aminoglycosides and carbapenems. Multiple drug resistance was defined as resistance to all 5 drug classes. A logistic model was used to test the association between resistance and hospital characteristics (two-sided p-value <0.05). Hospital characteristics included were type (university/teaching, general/secondary and other), presence of (neonatal) intensive care units (ICUs), and transplants, burns and heamatology units, number of beds, and number of ICU beds.
Results: A significant proportion of isolates was resistant against piperacillin (± tazobactam) (14.5%), ceftazidim (13.6%), fluoroquinolones (25.0%), aminoglycosides (19.7%) and/or carbapenems (16.7%). Multiple drug resistance occurred in 4.7% of all isolates, although proportions varied between countries. All multiple drug resistant isolates originated from hospitals with ICUs, although not all isolates originated from patients being treated at ICUs. Resistance rates in hospitals were associated with the number of intensive-care beds and presence of a neonatal ICU ($p < 0.0001$) only. None of the other hospital characteristics influenced the resistance rates.
Conclusions: An important proportion of PAE isolates was found resistant to multiple drugs. The proportion of multiple drug resistance was associated with the number of ICU beds and presence of a neonatal ICU. Hospital types (university, general, other) were not associated to PAE resistance, which might reflect differences in definition of hospital type.

O363 Resistance in *Pseudomonas aeruginosa* and *Acinetobacter* spp. from blood in the UK and Ireland, 2001–2005

R. Reynolds, R. Hope on behalf of BSAC Working Party on Bacteraemia Resistance Surveillance

Objective: The BSAC Bacteraemia Resistance Surveillance Programme monitors resistance to established and developmental antibiotics among the pathogens of bacteraemia in the UK and Ireland.
Methods: Between 2001 and 2005, 29 laboratories sent 1,014 *P. aeruginosa* and 200 *Acinetobacter* spp. for central MIC testing by BSAC methods. Ceftazidime, ciprofloxacin, gentamicin, imipenem and piperacillin-tazobactam (CAZ, CIP, GEN, IPM and TZP) were tested throughout. Tetracycline, minocycline and tigecycline (TET, MIN and TGC) were tested from 2002, ceftobiprole (BPR) from 2004, and doripenem (DOR) from 2005. Results were compared with LabBase2, a voluntary system taking routine results for blood isolates from nearly 400 centres in England, Wales and (from 2002) N. Ireland.
Results: Non-susceptibility in *P. aeruginosa* was 3% for CAZ, 7% for GEN, IPM and TZP and 19% for CIP; 8/1,014 (1%) were non-susceptible to all 5 agents, and 8% non-susceptible to ⩾2 classes of agents. LabBase2 results differed for CAZ and CIP (both 6% non-susceptible). For CIP, a lower breakpoint (set in 2005, and used for all BSAC isolates, but not in LabBase) explained the difference. Factors independently associated with increased risk of non-susceptibility to ⩾1 of these agents in *P. aeruginosa* were intensive care, young age, >48 hours prior hospitalisation, and infections arising from skin/soft tissue and lines.
Among *Acinetobacter*, resistance was concentrated in *A. baumannii*: 2/110 (2%) of *A. baumannii* were non-susceptible to all 5 agents, and 36% to ⩾2 classes of agents. Overall, ⩽5% of *Acinetobacter* were non-susceptible to GEN and IPM, and 17%, 27% and 63% to TZP, CIP and CAZ respectively. Numbers were too small to assess trend, predictors or agreement with LabBase2.
MIN and TGC largely overcame TET resistance in *Acinetobacter*, with maximum MICs of 8 and 4 mg/L respectively. DOR MICs were closely related to IPM, on average 2.8 dilutions lower for *P. aeruginosa* and 0.5 dilutions higher for *Acinetobacter*. BPR MICs were on average 1.2 dilutions higher than CAZ for *P. aeruginosa* and 2 dilutions lower for *Acinetobacter*.

Drug	*Pseudomonas aeruginosa*				*Acinetobacter* spp.			
	N	MIC mode	MIC_{90}	%NS (MIC)	N	MIC mode	MIC_{90}	%NS (MIC)
CAZ	1,014	1	4	3% (>8)	200	4	16	63% (>2)
CIP	1,014	0.25	4	19% (>0.5)	200	0.12	64	27% (>0.5)
GEN	1,014	1	4	7% (>4)	200	⩽0.12	16	5% (>4)
IPM	1,014	1	4	7% (>16)	200	0.06	0.25	2% (>4)
TZP	1,014	4	16	7% (>16)	200	⩽0.5	⩾512	17% (>16)
TET	Not tested – inherently resistant				156	2	⩾256	n/a
MIN	Not tested – inherently resistant				156	⩽0.06	2	n/a
TGC	Not tested – inherently resistant				156	0.25	2	n/a
BPR	442	2	8	n/a	81	0.5	32	n/a
DOR	226	0.06	0.5	n/a	35	0.06	0.5	n/a

MICs in mg/L. NS = non-susceptible (MICs in the range defined in brackets).

Conclusion: Most bloodstream *P. aeruginosa* in the UK and Ireland remain susceptible to relevant antibiotics. Carbapenem-resistant *Acinetobacter*, although widely referred to the HPA specialist laboratory from other infection sites, are rare in bacteraemia. TGC may be useful for *Acinetobacter* infections and BPR for *P. aeruginosa*; DOR has good activity against both species.

O364 Multidrug-resistant bacteria surveillance, France, 2002–2005

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Background: The prevalence rate of multidrug resistant bacteria (MDRB) in French hospitals is one of the highest among European countries. Since the mid-1990s, control of MDRB patient-to-patient transmission has becoming a main priority for the national infection control programme. In 1998, hospitals were advised to strengthen MDRB surveillance and prevention based on defined national guidelines.
Methods: To assess the impact of the control programme, a national coordination of MDRB surveillance networks was set up in 2002: data were collected three months a year from volunteer hospital laboratories. All diagnosis specimens (a strain with the same antibiotype per patient) of methicillin-resistant *Staphylococcus aureus* (MRSA), and Enterobacteriaceae producing extended-spectrum β-lactamase (ESBLE) were prospectively included. The incidence rate per 1000 patient–days (pd) was estimated for MRSA and ESBLE. Incidence between 2002 and 2005 was compared using the Poisson confidence interval.
Results: The number of participating laboratories has increased from 478 in 2002 to 589 in 2005. The incidence per 1000 pd was the highest in intensive care unit (2.24), compared to 0.76 in acute care and 0.39 in long-term care facilities. When all care units were considered, the incidence of MRSA decreased significantly from 0.63 in 2002 to 0.58 per 1000 pd in 2005. The decrease was observed mostly in acute care and intensive care units. The incidence of ESBLE increased significantly from 0.13 to 0.16 per 1000 pd. This increase was observed in all type of care facilities. *Enterobacter aerogenes* was the most frequent ESBLE (36%), in 2002 and *Escherichia coli* (39%) in 2005.
Conclusion: These results demonstrates the positive impact of the national prevention programme on hospital-acquired MRSA rates. However, the incidence remains high and efforts have to be sustained. In contrast, incidence of ESBLE is increasing, especially ESBL *Escherichia coli*, which could be a threat for the community.

O365 10 years of surveillance of bloodstream infections in European medical centres by the SENTRY Antimicrobial Surveillance Program (1997–2006)

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Objectives: We assessed the frequency of occurrence and antimicrobial susceptibility (S) of pathogens causing bloodstream infection (BSI) in European (EU) medical centres participating in the SENTRY Program.
Methods: The first 20 unique and clinically relevant BSI isolates were collected from 44 medical centres (10–31/year) located in 16 EU countries, Turkey and Israel and sent to a central monitor each month. Isolates were tested for S by broth microdilution methods and results interpreted according to the 2006 CLSI M100-S16 document. S rates by country and variations over time were analysed.
Results: A total of 47,621 strains were processed. The antimicrobial S rates of the most common Gram-negative bacilli are shown in the table. The most common Gram-positive cocci were (rank order among all/no. of isolates/%of total): *Staphylococcus aureus* (2/9,291/19.5%) > coagulase-negative staphylococci (3/6,202/13.0%) > *Enterococcus* spp. (4/3,598/7.6%). The rank order and the frequencies were stable over the interval. MRSA rates varied from 0.7% in Sweden to 47.5% in the UK and 48.2% in Israel, but remained very stable over time; while VRE rates varied from <1.0% in France and Spain to >14% in Ireland and UK. In general, VRE increased from 3.2% in the 2001–2002 to 7.2% in the 2005–2006 period. ESBL phenotypes varied from 0.8% in Sweden to 24.0% in Turkey among *Escherichia coli* (5.2%, all EU), and from 1.0% in Switzerland to 40.6% in Israel and >50% in Greece among *Klebsiella* spp. (KSP; 21.9%, all EU). ESBL phenotypes among *E. coli* and KSP increased from 3.7 and 19.8% in 1997–1998 to 6.8 and 23.7% in 2005–2006, respectively; while S to ciprofloxacin (CIP) among *E. coli* and KSP decreased from 91.9% and 94.4% in 1997–1998 to only 80.3% and 84.6% in 2005–2006, respectively. Decreased S to imipenem (IMI; MIC ⩾ 8 mg/L) varied from <3% in Sweden and UK to 32.2% in Turkey and 37.9% in Greece among *P. aeruginosa*, and from 0% in Germany, Sweden and Switzerland to 40.3% in Greece and 48.2% in Turkey among *Acinetobacter* spp. In general, S to IMI dropped from 84.8% and 80.3% in 1997–1998 to 79.0% and 66.7% in 2005–2006 among PSA and ASP, respectively, due to metallo-β-lactamases.

Rank order	Organism (no. tested/% of total)	MIC_{90} / % Susceptible			
		Cipro-floxacin	Gentamicin	Cefepime	Imipenem
1	*E. coli* (11,036 / 23.2)	4 / 85.6	⩽2 / 94.1	⩽0.12 / 98.2	⩽0.5 / >99.9
5	*Klebsiella* spp. (3,470 / 17.3)	2 / 89.2	>8 / 85.2	8 / 91.4	⩽0.5 / 99.5
6	*P. aeruginosa* (2,965 / 6.2)	>4 / 71.1	>8 / 72.3	>16 / 76.1	>8 / 79.7
7	*Enterobacter* spp. (2,038 / 4.3)	>4 / 82.5	8 / 89.1	4 / 96.2	1 / 99.2
9	*Acinetobacter* spp. (1,203 / 2.5)	>4 / 39.9	>8 / 41.3	>16 / 48.1	>8 / 73.0
12	*Proteus mirabilis* (892 / 1.9)	>4 / 81.4	>8 / 85.2	0.25 / 95.7	2 / 99.7

Conclusions: The following R phenotypes showed significant increase during the study period: VRE, ESBL and CIP R among *E. coli* and KSP, and IMI R among PSA and ASP. A wide geographic variation was observed; with higher R rates being more frequent in UK, Ireland and southeastern countries and lower rates in Sweden and Switzerland.

O366 Plasmid-mediated quinolone resistance in non-typhi serotypes of *Salmonella enterica* in Ireland in 2001–May 2006

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Objectives: To screen non-typhoid *Salmonella enterica* isolates for plasmid encoded quinolone resistance determinant qnrA, qnrB and qnrS.
Methods: All 5340 isolates of non-typhoid *Salmonella enterica* received from humans and animals between 2001 and May 2006 were included. Susceptibility to nalidixic acid and ciprofloxacin was performed in accordance with the disk diffusion methods of the Clinical Laboratory Standards Institute. Ciprofloxacin MICs were determined by Etest for all nalidixic acid resistant isolates, and isolates with an MIC of >0.25 ug/mL were screened for the presence of qnrA, qnrB and qnrS by PCR using specific primers previously described. A positive control for qnrA was included and all samples were confirmed as amplifiable using universal primers for 16SrRNA.
Results: Eighty-one (1.5%) isolates had a ciprofloxacin MIC > 0.25 ug/mL. The qnrA positive isolate yielded a product of expected size. All isolates tested were negative for the qnrA gene. One isolate of *Salmonella enterica* ssp. *houtenae* (IV or 4) tested positive for qnrB on PCR and sequencing confirmed a qnrB 5. The qnrS gene was identified in 9 salmonella isolates. Of these, 4 were serovar (S.) Corvallis, 2 S. Virchow, 1 S. Typhimurium, 1 S. Enteritidis and 1 S. Schwarzengrund. Sequences for 8 isolates were 100% homologous to published sequences for qnrS1 and 1 isolate (S. Virchow) differed from the published qnrS sequence by a single nucleotide. A history of foreign travel was reported for one isolate (the S. Schwarzengrund).
Conclusion: The plasmid mediated quinolone resistance determinants qnrB (1) and qnrS (9) were identified in 10 of 81 *Salmonella enterica* isolates with raised ciprofloxacin MICs. S. Corvallis is an uncommon serovar in our experience (0.2% of all isolates) and is remarkable that this serovar represents a high proportion of all qnrS positive isolates detected. There is no evidence of an epidemiological link between the S. Corvallis isolates.

O367 Significant increase in isolation of ESBL-producing and ciprofloxacin-resistant non-typhoidal Salmonellae from Pakistan

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Introduction: Increasing antimicrobial resistance in non typhoidal Salmonellae (NTS) is a global challenge. Data regarding quinolone and 3rd-generation cephalosporin resistance in NTS is limited from developing countries.
Material and Methods: This descriptive study was conducted from 1990 to 2006 at Aga Khan University, Karachi. All clinical samples yielding the growth of NTS were retrieved from computerised data base and included in the study. Duplicate samples from the same patient were excluded. During the study period NTS were isolated and identified using standard microbiological techniques. Antimicrobial susceptibility testing was performed by Kirby Bauer method according to Clinical Laboratory Standard Institute (CLSI). Extended spectrum β-lactamase production (ESBL) was detected using combined disc method according to CLSI. Sensitivity to ciprofloxacin was detected by nalidixic acid screening method. Minimum inhibitory concentration (MIC) of ciprofloxacin was determined by agar dilution method according to CLSI. Statistical analysis was performed using SPSS version 13.
Result: During the study period of 16 years 1,651 NTS were isolated. Out of these 1,519 (92%) were from stool and 114 (8%) were from blood and sterile sites. Most prevalent were *Salmonella* group B (579), followed by non-typeable *Salmonella* spp. (709). Isolation of NTS was high in children less than 5 years of age (58.5%). Ciprofloxacin-resistant NTS (MIC $\geqslant 0.25$) increased from 29% in 2002 to 49% in 2006. Ceftriaxone-resistant NTS increased from 2% in 2000 to 9% in 2006. ESBL production was seen in 98.5% (n = 68) of the ceftriaxone-resistant NTS. ESBL positive isolates exhibit high resistance against amoxicillin clavulanic acid (57%), gentamicin (58%) and amikacin (23%). No resistance was seen to piperacillin tazobactam and imipenem. Interestingly resistance against the first-line drugs remained stable over the years. Rather resistance against chloramphenicol decreased from 26% in 1990 to 2% in 2006. Similarly resistance against cotrimoxazole and ampicillin remained static with minimum variations. Moreover multidrug resistant NTS strains decreased from 26% in 2002 to 0% in 2006.
Conclusion: Increase in drug resitant NTS is a serious threat to public health and it necessitates continuous surveillance and use of appropriate screening tests for laboratory detection. Decrease in antimicrobial resistance against first line agents suggests decrease utilisation of these drugs in the community.

The role of rapid diagnostics for patient screening: new tools in the fight against *Staphylococcus aureus* and MRSA

S372 A surgeons perspective on staphylococcal infection

R. Morgan-Jones, J. Kluytmans, M. Bonten (Cardiff, UK; Breda, Utrecht, NL)

Orthopaedic and Trauma surgery is an implant specialty where surgical success is related to the insertion of prosthetic joints (e.g. knee, hip and shoulder replacements) and reconstructive metalwork following trauma (e.g. intra-medullary nails, plates and screws).
The worst complication is infection which results in multiple additional operations, extended hospitalisation, prolonged morbidity and poor functional outcomes. Infection rates are low, ideally between 0.5% and 2%. The most common infective organism is *Staphylococcus aureus* which accounts for 50–70% of cases. Methicillin-resistant coagulase-negative staphylococci are of growing importance but numerically less significant. The clinical presentation, surgical techniques and pre/post operative morbidity of chronic osteomyelitis and infected joint replacement will be discussed to project the patients' perspective of *S. aureus*.

S373 *Staphylococcus aureus*: effect of perioperative eradication of carriage

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Staphylococcus aureus is the leading nosocomial pathogen in hospitals all over the world. It is associated with substantial morbidity and mortality that is increasing due to the widespread dissemination of methicillin-resistant *S. aureus*. It is important to prevent infections with *S. aureus*. Control of *S. aureus* infection has been based traditionally on the prevention of cross-infection. However, in recent years it has been shown repeatedly that the majority of nosocomial *S. aureus* infections originate from the patients' own flora. Nasal carriage of *S. aureus* at admittance to the hospital is now considered a well-defined risk factor for subsequent infection in various groups of patients, including patients undergoing surgical procedures. The relative risk of carriers to develop a surgical site infection is approximately 8 times higher than non-carriers. Also, when carriers of *S. aureus* develop an infection after surgery, the strains from the infection are identical to the strains carried before surgery in more than 80% of the cases.
Based on these findings several intervention studies have been performed. Although the results were promising, final conclusions could not always be made due to methodological deficiencies. Most studies used historic control groups and included both carriers and non-carriers. Subsequently, a number of randomised controlled trials were performed. In general, the results of these studies were showing a trend towards a beneficial effect of mupirocin but failed to produce significant results. Proposed explanations are mainly: (1) the infection rates in the placebo groups were lower than anticipated. Therefore, the sample size was too low. (2) Due to the duration before the results of culture are available, carriers could not be identified before inclusion. Therefore both carriers and non-carriers were included resulting in a dilution of the effect.
A systematic review was performed to determine the effect on the *S. aureus* infection rate of treating identified carriers of *S. aureus* with mupirocin nasal ointment. Only prospective and adequately controlled trials were included. The literature search resulted in eleven hits. Only three articles met the inclusion criteria. Among the 556 mupirocin-treated surgical patients with *S. aureus* nasal carriage there were 20 *S. aureus* infections (3.6%), as compared with 7.5% (42/559) in the controls (nasal carriers without mupirocin treatment) [p = 0.006; Number Needed to Treat (NNT) = 25]. Prophylactic intranasal mupirocin significantly reduced the rate of *S. aureus* infections among surgical patients who were *S. aureus* carriers. Prospective studies in carriers only are needed to determine the overall effectiveness of mupirocin in surgical patients.

S374 Modelling of MRSA: the potential benefits of rapid diagnostic testing

M. Bonten (Utrecht, NL)

Mathematical modelling is a tool to describe the complex dynamics of MRSA epidemiology. A recently published model (Bootsma et al. PNAS 2006) will be presented. In this model the effects of the individual components of the Dutch 'Search & Destroy' policy are evaluated, both in settings of low and high MRSA-endemicity. A consequence of this policy is the pre-emptive isolation of patients suspected of MRSA-colonisation. With conventional cultures it can take up to 5 days before colonisation can be ruled out. The effects of rapid diagnostic testing (with different test characteristics) on the number of isolation days needed will be presented.

Emerging viral zoonotic diseases

S390 Emerging pathogens and host species barriers

T. Kuiken (Rotterdam, NL)

Emerging infectious diseases have a major impact on public and animal health, the economy, and the environment (Science 309: 1680). Human

mortality from recently emerged diseases varies, ranging from less than 200 people thus far for H5N1 avian influenza to about 20 million for AIDS. Livestock production has been negatively affected by the direct mortality of animals from emerging infections and depopulation policies to protect the safety of international trade and to control the spread of pathogens. The environmental impact of emerging infections is of special concern for endangered wild animal populations, which can be pushed to the brink of extinction by such events. Animals, and particularly wild animals, are thought to be the source of more than 70% of all emerging infections (Philos Trans R Soc London B 356: 983). Understanding how some infectious agents have breached the barriers that normally limit this interspecies transmission is important. However, these barriers are poorly characterised. The host species barrier for infection can be defined as an interaction of factors that collectively limits the transmission of an infection from one host species (the donor species) to another (the recipient species) (Science 312: 394). Such limiting factors may occur at different levels, of which the functional components are organism, tissue, and cell. At the organism level, barriers limiting contact between two host species, and thus preventing transmission of an infection, may be geographical, environmental, or behavioural. Because of the rapid growth of both human populations and their consumption patterns, these barriers are often breached. Even if two host species share the same geographical area and habitat, pathogen transmission may not occur due to behavioural restrictions. At the tissue level, an emerging pathogen, in particular a virus, needs to gain access to the appropriate tissue or tissues in order to replicate. First it must invade the host through a portal of entry. It may then remain localised and replicate in a tissue near the original portal of entry, or may generate a systemic infection, using various pathways to spread to more distant target tissues in the host. Release of progeny virus from the infected host typically occurs through dissemination by respiratory, enteric, or urogenital secretions, or by an arthropod vector that has ingested blood from a viremic host. There are barriers at each stage of this process that may prevent the virus from developing a productive infection. At the cellular level, a virus needs to enter, replicate in, and exit from the appropriate cell type, simultaneously dealing with any host response to infection. Understanding how a pathogen is able to breach the constraints of the host species barrier and become established in a new host species will help us to detect and control the emergence of zoonotic infections.

S391 The domestic animal revolution and influenza

B. Olsen (Kalmar, SE)

In humans, influenza A virus causes yearly seasonal influenza epidemics of respiratory disease resulting in high morbidity. There are many different subtypes of influenza virus which are characterised according to two surface structures – the hemagglutinin (H1–H16) and neuraminidase (N1–N9). These subtypes have the ability to recombine, thereby creating new variants, for example H5N1. If a subtype that humans have not encountered starts to spread it can result in a pandemic. Pandemic outbreaks have occurred at irregular intervals and have had a devastating impact on mankind. The Spanish influenza pandemic of 1918 is thought to have killed about 50 million people. In particular dabbling ducks are believed to constitute the main reservoir for influenza A virus. In ducks, the virus causes sub-clinical gastrointestinal infection. By a meta-analysis on all published screening data of wild birds and data from a four-year screening of ducks caught and sampled in Sweden we found that the prevalence of influenza A virus in the western Eurasian duck population shows a different temporal pattern compared to North America. The prevalence in western Eurasian ducks is high from August to December and also rises in the spring. These findings are of importance for the understanding of how influenza virus is perpetuated in nature. Of concern is the presence of H5 and H7 subtypes that are prone to change into highly pathogenic variants in poultry. Many of the strains isolated in our study are prototypes that have caused influenza outbreaks in poultry in Europe during recent years. This indicates that wild bird surveillance for influenza A virus can be of value as a sentinel system to prevent outbreaks in domestic poultry.

A result of the domestic animal revolution is that there has never before in history been so much poultry as today. This fact, in combination with a growing number of humans, creates an arena where domestic animals, humans and finally zoonotic pathogens can interact. Influenza A virus is the ultimate actor in this play where some subtypes may change into highly pathogenic forms. These may be transmitted directly to man. Another worrying scenario is that an avian influenza virus will reassort with a circulating human influenza. A genetic trait might then readily go from the human to the bird virus so that avian influenza acquires the capacity to pass from person to person. With this risk in mind, it would advisable to consider any method to reduce the probability of this happening. Contingency planning should take into account the known evolutionary potential of animal viruses and other pathogens to adapt to the environment of humans and domestic animals.

S392 Wildlife hosts of SARS-coronavirus and related viruses

L-F. Wang (East Geelong, AU)

Severe acute respiratory syndrome (SARS) represents the first pandemic transmissible disease of previously unknown aetiology in the 21st century. The pandemic started in November 2002 in Guangdong, China and was brought under control in July 2003, after it had spread to 33 countries on 5 continents resulting in more than 8000 infections and close to 800 deaths. The outbreak was caused by a newly emerged coronavirus, now known as the SARS coronavirus (SARS-CoV). A closely related virus was detected in masked palm civets from a live animal market in Guangdong during the outbreaks. In late 2003 and early 2004, sporadic outbreaks were reported in the same region of China where the 2002/3 outbreaks originated. This time, civets were identified as the direct source of the virus responsible for human infections. However, a large-scale survey in China failed to find any evidence of infection of farmed or wild civets with SARS-CoV. It appears that the infection of civets was limited to animals present in the live animal markets only. The role of civets as a natural reservoir of SARS-CoV was hence never confirmed. Molecular epidemiological studies revealed that the viruses responsible for the 2003/4 outbreaks were not the same as those isolated during the 2002/3 outbreaks, indicating independent species-crossing events. These findings indicate that a SARS epidemic may recur in the future and that SARS-like coronaviruses (SARS-like-CoVs) originating from different reservoir host populations may lead to epidemics at different times and in different regions depending on the distribution of the reservoirs and transmitting hosts. This notion was supported by the later discovery of a group of diverse SARS-like-CoVs in bats. At least five different SARS-like coronaviruses have been identified in different species of horseshoe bats in the genus *Rhiolophus*. Sequences of four complete genomes were determined and the overall nucleotide sequence identity between bat SARS-like coronaviruses and SARS-CoV was 87–92%. Although the exact host species for the SARS-CoV has not been identified, the great genetic diversity of bat coronaviruses highly suggests that a different horseshoe bat or a closely related bat species is the most likely natural reservoir of the SARS-CoV responsible for the 2002–2004 outbreaks.

S393 Bats: important hosts of emerging viruses

C. Calisher (Fort Collins, US)

Bats (order Chiroptera, suborders Megachiroptera and Microchiroptera) are abundant, diverse, and geographically widespread. Bats represent about 20% of the approximately 4,600 species of mammals. The megachiropterans include 166 species and the microchiropterans 759 species. Bats provide us with resources but their importance is minimised and many of their populations and species are at risk, even threatened or endangered. Some of their characteristics (what they eat, whether they are colonial or solitary, how their populations are structured, that they can fly, that they migrate seasonally and may move daily, that they enter torpor and/or hibernate, that they are able to live for many years, that they have peculiar roosting behaviours, that they can echolocate, and that they have various virus susceptibilities) make them

exquisitely suitable hosts of viruses and other disease agents. Bats of certain species are well recognized as being capable of transmitting Rabies virus but recent observations of outbreaks and epidemics of newly recognized human and livestock diseases caused by viruses transmitted by various megachiropteran ("flying foxes") and microchiropteran bats have drawn attention anew to these remarkable mammals. This presentation summarises information regarding chiropteran characteristics and information regarding 66 viruses [at the time of preparation of this abstract] that have been isolated from bats. It is clear that we do not know enough about bat biology, that we are doing too little in terms of bat conservation, and that there remain a multitude of questions regarding their role in disease emergence. Recent observations have proven these points and will be discussed.

Controversies in fungal disease

S395 Challenges and progressions in antifungal susceptibility testing

S. Arikan (Ankara, TR)

Development of standard antifungal susceptibility testing (AFST) assays has been one of the most important progressions in the field of medical mycology. CLSI (Clinical and Laboratory Standards Institute) microdilution and disk diffusion methods as well as AFST-EUCAST (European Committee for Antimicrobial Susceptibility Testing – Antifungal Susceptibility Testing Subcommittee) microdilution reference methodology are the currently used standard assays. Recent progressions have further expanded the knowledge on application and clinical utility of AFST. Among these are validation of the previously documented CLSI fluconazole MIC breakpoints and dose-dependent susceptibility concept for fluconazole vs. *Candida*, and documentation of MIC breakpoints and disk diffusion inhibition zone interpretive parameters for voriconazole vs. *Candida*. The use of fluconazole susceptibility profile as a surrogate marker for prediction of voriconazole susceptibility has also been proposed recently. In addition, based on the modifications of the CLSI M38-A reference method, new guidelines for in vitro susceptibility testing of antifungal agents against *Aspergillus* spp. have been developed by AFST-EUCAST. A standard assay for testing antifungal agents against dermatophytes is also under development. Further investigations address the applicability of other methods, which are more practical and/or require shorter incubation periods. Among these are Etest, colorimetric methods, flow cytometry, and ergosterol quantitation. Utility of flow cytometry for AFST of yeasts and moulds is being currently studied and appears promising. Assessment of metabolic activity by using XTT colorimetric assay is also being investigated as a novel approach, particularly for determination of quantitative endpoints for testing caspofungin against *Aspergillus*, and for susceptibility testing of Zygomycetes. Despite these progressions, utility of AFST in direction of antifungal therapy and prediction of clinical outcome is still limited. While in vitro triazole (particularly fluconazole) susceptibility results for *Candida* appear to be optimally correlated with clinical outcome, data are either limited and investigational or fail to demonstrate any significant in vitro–in vivo correlation for most of the remaining fungus–antifungal drug combinations. Importantly and conclusively, the results obtained by AFST constitute one of the several factors that influence clinical outcome. Optimisation of test methodologies and parameters for routine use and expanded in vitro-in vivo correlation studies may further enhance the role of AFST as an adjunct in direction of antifungal therapy.

Harmonising expert rules and interpretive reading for antimicrobial susceptibility testing in Europe

S398 The concept of interpretative reading of the antibiogramme

P. Courvalin (Paris, FR)

In recent years, there has been important progress in our knowledge of bacterial resistance to antibiotics. The availability of a large number of antibiotics has allowed precise individualisation of resistance phenotypes, and enzyme inhibitors have provided clues concerning certain mechanisms of resistance. Detailed analysis of the bacteriostatic and bactericidal activity of antibiotics, alone or in combination, has indicated the limits of in vitro antimicrobial susceptibility tests in the detection of resistance resulting in clinical failure. The goal of combined molecular and therapeutic interpretation of susceptibility tests is to provide an improved logical basis for decision-making in antibiotic therapy by taking into account the recent progress in the understanding of bacterial resistance. The molecular analysis and therapeutic interpretation, designated "interpretative reading", of the in vitro antibiotic susceptibility tests consists of three steps: (1) characterisation of the resistance phenotype with a judicious assortment of antibiotics belonging to the same class; (2) deduction from the observed phenotype of the corresponding biochemical mechanism of resistance; and (3) inference from the deduced mechanism of the predicted resistance phenotype. In vitro antibiotic susceptibility tests, like other tests in biology, should provide objective quantitative data, e.g. MICs. For various historical reasons, they also provide subjective interpretation of the data such as clinical categories. Interpretative reading of antimicrobial susceptibility tests is an attempt to reconcile these two notions by basing interpretation on the most recent knowledge in the field of antibiotic study, in particular that of mechanisms of resistance. The best that one can ask of antibiotic susceptibility testing is detection of resistance, in particular of low-level resistance. This can be achieved by improved interpretation of the results of in vitro sensitivity tests or by the design of certain genotypic approaches. The goal of the proposed approach is to provide the clinician with the necessary results for judicious decision-making in antibiotic therapy utilising available information and to draw his or her attention to the combinations of bacterium and antibiotic for which there is a therapeutic risk.

S401 Expert rules for aminoglycosides, macrolides and lincosamides

R. Leclercq (Caen, FR)

Resistance to aminoglycosides is mostly related to production of modifying enzymes, phosphorylases, nucleotidyltransferases or acetyltransferases. The enzymes vary in their substrate ranges which are often broad and each enzyme is characterised by a particular profile of resistance which allows its putative identification from the conferred phenotype. The enzymes from Gram-positive cocci generally differ from the enzymes detected in Gram-negative bacteria. Generally, the presence of an enzyme confers to the host frank resistance to the antibiotics modified in vitro. However, there are some exceptions, both in Gram-negative and in Gram-positive bacteria. For instance, resistance of Gram-positive organisms to the kanamycin–neomycin group of antibiotics is due to the synthesis of an APH(3′)-III enzyme. The enzyme catalyzes efficient phosphorylation of amikacin in cell-free extracts but does not always determine resistance to this antibiotic. However, bactericidal synergism of amikacin with β-lactams or vancomycin is always abolished. Therefore, the test of kanamycin better predicts the activity of amikacin against Gram-positive organisms. In this case and several others, interpretive reading of susceptibility tests based on identification of resistance phenotypes may help to identify impaired activity of aminoglycosides.

Interpretive reading may also allow to identify some pitfalls in the detection of resistance to macrolide and lincosamide antibiotics. Staphylococci may be resistant to macrolides by production of a ribosomal methylase encoded by erm genes conferring the MLSB phenotype or by production of an efflux pump encoded by the msr(A) gene. In case of inducible MLSB resistance, clindamycin that is not an inducer remains active. However, constitutively resistant mutants can be selected by clindamycin and clinical failure during treatment by clindamycin have been reported in a few occasions. The use of clindamycin is probably best avoided in severe infections or infections with heavy bacterial inocula. In case of resistance by efflux, clindamycin is not substrate for the pump and the risk for selection of resistant mutants is not greater than that for erythromycin-susceptible isolates. By a disk diffusion test, the inducible MLSB phenotype can be identified by the flattening of the clindamycin zone facing the erythromycin disk.

Challenges in infection control

S404 *Pseudomonas aeruginosa*: don't go near the water

M. Dettenkofer (Freiburg, DE)

P. aeruginosa is amongst the most problematic nosocomial pathogens, especially in intensive care settings. The organism has a predilection for moist environments ("don't go near the water" – subtitle of an editorial by M. Bonten and R. Weinstein in Crit Care Med 30, 2002): aqueous solutions, e.g. soaps, irrigation fluids, eye drops, and dialysis fluids, may become contaminated with *P. aeruginosa*. It is frequently detected in faucet aerators and traps of sinks and in case of inadequate reprocessing even in respiratory therapy equipment. *P. aeruginosa* may contaminate bronchoscopes leading to severe outbreaks. In case of long or artificial fingernails, healthcare personnel may harbour *P. aeruginosa* which may be associated with outbreaks caused by *P. aeruginosa*. Finally, the organism may be found on the surface of raw fruits or vegetables. Within hospital settings, colonised patients, especially those frequently treated with broad-spectrum antibiotics, are important reservoirs.

The relative importance of the contaminated inanimate environment is discussed controversially: Whereas outbreaks due to environmental sources have been described repeatedly, its role as a source for endemic colonisation (and, as a consequence, infection) in intensive care patients has not been firmly established. More recent data show that in an ICU, a high figure of 35% of all cases of *P. aeruginosa* acquisition may originate from contaminated tap water (clonal relationship between isolates) and that retrograde contamination of faucets by patients may occur in 15%. However, surveillance of intestinal colonisation (which plays a relevant role in the epidemiology of *P. aeruginosa*) was not undertaken. The majority of colonised patients is already colonised on admission, with preceding antibiotic exposure being responsible for the majority of cases of acquired respiratory tract colonisation.

With regard to infection control, adequate hand hygiene is essential, whereas 'sterilisation' of sinks or routine use of water filters seems impractical. Alcohol-based handrubs, via dispensers near the bedside or in coat pockets, should be preferred in most situations over hand washing with soap and water. All open water sources including sinks may be a potential habitat of pathogens like *P. aeruginosa*, and the use of tap water for critically ill or immuno-compromised patients must be restricted.

Nosocomial urinary tract infection

S406 Nosocomial urinary tract infections in urology sections. Data from the PEP and PEAP-studies

T. Bjerklund Johansen, M. Cek, K. Naber, M. Grabe, P. Tenke on behalf of the European Society for Infection in Urology

Introduction and Objectives: We wanted to study the etiology, prevalence and diagnostic practice related to nosocomially acquired urinary tract infections (NAUTI) in urology departments. The results are presented on behalf of the PEP and PEAP-study investigators.

Material and Methods: Two internet based prevalence registrations were carried out in November 2003 and 2004 respectively. 152 hospitals from Europe and Asia took part and 6033 hospitalised urology patients were screened for NAUTI. Detailed information on 727 patients with NAUTI were provided.

Results: The most common pathogen was *Escherichia coli* accounting for 33% followed by *Pseudomonas* sp. in 14% and *Enterococcus* in 11%. A second pathogen was reported in 65% of patients and *Candida* sp. was seen in 14%. There were significant regional variations in the distribution of pathogens and the number of culture tests taken (0–5.9 cultures per patient admission). The principal pathogen was resistant to the most commonly used antibiotics in 60–90% of cases. The prevalence of asymptomatic bacteriuria was about 30% in most regions, while the prevalence of urosepsis varied between 2–27% in the regions studied.

Conclusions: Urology sections should be encouraged to monitor the susceptibility of pathogens causing NAUTI in order to tailor a better empirical antibiotic treatment. Urologists need to work out guidelines on when to take blood cultures after urological surgery to obtain a more uniform reporting of urosepsis between regions. The high prevalence of urosepsis after urological surgery is a cause of concern.

S407 Pathogens and resistance

T. Matsumoto (Kitakyushu, JP)

Escherichia coli accounted for about 80% of organisms in uncomplicated UTIs. In contrast, in complicated UTI many kinds of enterobacteriaceae isolates, staphylococci, enterococci, *Pseudomonas aeruginosa*, and the other glucose-nonfermentable Gram-negative rod (NFGNR) are isolated. Almost nosocomial UTI (NUTI) is complicated UTI. In the 2003 GPIU study (formerly PEP study), NUTI prevalence rate was 9.4% (326/3350). In the 2003 GPIU study, *E. coli* was the most frequent pathogen, accounting for about 30% of all pathogens isolated. *Pseudomonas* (13%), *Klebsiella* (10%), *Enterococcus* (9%), and *Proteus* (7%) were isolated frequently. In our hospital, *Enterococcus faecalis* was the most frequent of all isolated organisms, accounting for 24%. The other enterococci (12%), *S. marcescens* (12%), *P. aeruginosa* (9%), the other NFGNR (9%), *E. coli* (6%), *Staphylococcus epidermidis* (6%) were isolated frequently. It is considered that these differences occurred by the difference of patients' background.

The fluoroquinolone- and cephem-resistant enterobacteriaceae isolates from patients with NUTI are increasing. Most of the cephem-resistant isolates of *E. coli*, *K. pneumoniae* and *P. mirabilis* are producing Extended-Spectrum β-lactamases (ESBLs). ESBLs are plasmid-mediated broad-spectrum β-lactamases. The ratios of ESBL producers in *E. coli* and *K. pneumoniae* are quite different in each country (less than 5% to more than 80%). Most of the nosocomial ESBL producers have acquired resistance to non-β-lactams, such as fluoroquinolones, phosphomycin, co-trimoxazole. Some of the multi-drug resistant isolates have no effective oral antibiotics. According to the 2003–2004 GISP study, the ratios of fluoroquinolone resistance of *E. coli* and *K. pneumoniae* accounted for about 20%, respectively, that of enterococci accounted for more than 60%. Mechanisms of resistance to quinolones are mainly target mutations, especially GyrA and ParC. Qnr that is plasmid encoded quinolone resistance reported in 2002. The emergence of qnr also alerts us to the potential rapid dissemination of quinolone-resistant determinants. Qnr shows quinolone specific resistance, but the qnr-plasmids reported are integron-associated and carry multiple resistance determinants providing resistance to several classes of antimicrobials including β-lactams and aminoglycosides.

Multi-drug resistant isolates cause nosocomial spread easily. There are many reports about nosocomial spread of multi-drug resistant ESBL producers. To prevent prevalence of antimicrobial-resistant isolates appropriate antimicrobial selection is needed. Geographic variations in pathogen occurrence and susceptibility profiles require monitoring continuously, and it is important to update treatment guidelines based on susceptibility profile and the results of clinical trials.

S409 Treatment options in nosocomial urosepsis

F. Wagenlehner (Straubing, DE)

Urosepsis accounts for approximately 25% of all sepsis cases and may develop from a community or nosocomial acquired urinary tract infection (UTI). The underlying UTI is almost exclusively a complicated one with involvement of the parenchymatous urogenital organs (e.g. kidneys, prostate) and mostly associated with any kind of obstructive uropathy. If urosepsis originates from a nosocomial infection, a broad spectrum of Gram-negative and Gram-positive pathogens have to be expected which are often multiresistant.

In urosepsis, as in other types of sepsis, the severity of sepsis depends mostly upon the host response. The treatment of urosepsis follows the generally accepted rules of the Surviving Sepsis Campaign Guidelines. Early normalisation of blood pressure and early adequate empiric antibiotic therapy with optimised dosing are equally important to meet the requirements of early goal directed therapy. In most cases of urosepsis an early control of the infectious focus is possible and as important. Optimal supportive measures need to follow the early phase of resuscitation.

Although most antibiotics achieve high urinary concentrations there are several unique properties in complicated UTI, and thus in urosepsis, that influence the activity of the antibiotic substances: (i) The renal pharmacokinetics in unilateral and bilateral renal impairment and in unilateral and bilateral renal obstruction differ. (ii) Varations in pH may influence the activity of certain antibiotics. (iii) Biofilm infection is frequently found under these conditions, which may increase the minimal inhibitory concentrations (MIC) of the antimicrobials at the site of infection by several 100-folds. In order to assess the antibiotic pharmacodynamic properties in such situations not only the MIC as determined in vitro and the plasma concentrations of the free (unbound) drug, which are the guiding principle for many infections, but also the actual renal excretion and the urinary bactericidal activity of an antibiotic substance should be taken into account. In the treatment of urosepsis it is important to achieve optimal exposure to antimicrobials both in plasma and in the urinary tract. The role of drugs with low renal excretion rate is therefore limited.

Since urosepsis originates quite often from catheter associated UTI and after urological interventions, optimal catheter care and optimal strategies to prevent nosocomial UTI may be able to reduce the frequency of urosepsis.

Community-acquired bacterial infection II

O410 Role of "atypical" respiratory pathogens in patients with exacerbations of chronic obstructive pulmonary disease

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Objectives: The term "atypical pathogen" commonly refers to *M. pneumoniae*, *C. pneumoniae* and *L. pneumophila*. Serological studies suggest that these pathogens may play a role in acute exacerbations of chronic obstructive pulmonary disease (AECOPD). We investigated the presence of atypical pathogens in sputum samples in patients with stable COPD and those with AECOPD using real-time PCR.

Methods: From May 1999 through March 2000, COPD patients (GOLD stage I and II) were recruited from the outpatient pulmonary clinic of the Medisch Spectrum Twente, a 1150-bed teaching hospital in Enschede, The Netherlands. Sputum samples were retrieved from patients with stable disease and during exacerbation. Sputum was processed with the NucliSens® easyMAG™ platform, (bioMérieux) and DNA was stored at −20ºC until processing was performed. For the detection of *M. pneumoniae* an assay based on the the PI adhesin gene was used. For the detection of *C. pneumoniae* an assay based on the ompA gene of *C. pneumoniae* was used. For the detection of *Legionella* two assays were used, targeted at specific regions within the 5S rRNA and the mip gene.

Results: A total of 248 sputum samples from 104 patients were analysed (mean age 63 years; range 45–75 years; 86 men, 18 women). In total, 122 stable-state sputa and 126 exacerbation sputa were available for testing. Of the stable-state sputa, all samples were negative for *M. pneumoniae* and *C. pneumoniae* DNA, whereas one sample was positive for *Legionella* non-pneumophila DNA. Of the exacerbation sputa, all samples were negative for *M. pneumoniae* and *C. pneumoniae* DNA, whereas one sample was positive for *Legionella* non-pneumophila DNA. In both *Legionella*-positive samples *S. pneumoniae* was cultured a level of growth of $>10^5$ cfu/mL.

Conclusion: We investigated the possible relationship between presence of atypical pathogens in patients with stable COPD and in those with AECOPD using real-time PCR and found no indication for an important role of *Legionella* spp., *C. pneumoniae* or *M. pneumoniae* in COPD. This study stresses the biases that nonstandardised serology may introduce in the context of the possible link between atypical pathogens and AECOPD.

O411 Community-acquired pneumonia caused by *Pseudomonas aeruginosa*: incidence, risk factors, and outcome

C. Garcia-Vidal, L. Muñoz, A. Mykietiuk, R. Verdaguer, J. Carratalà, F. Gudiol (Barcelona, ES)

Objectives: We sought to determine the incidence, risk factors and outcome of community-acquired pneumonia (CAP) caused by *Pseudomonas aeruginosa*.

Methods: Prospective observational study of non-severely immunocompromised adults hospitalised with CAP from 1995 through 2005. Patients with HIV infection, transplantation, and neutropenia were not included. A comparison between cases of CAP caused by *P. aeruginosa* and the remaining cases was performed.

Results: We documented a total of 2,455 consecutive cases of CAP, 21 (0.9%) of which were caused by *P. aeruginosa*. Four cases were polymicrobial (*Haemophilus influenzae* 2, *Streptococcus pneumoniae* 1, and *Legionella pneumophila* 1). Microbiologic diagnosis was established by one or more of the following methods: blood cultures 9, Gram stain and sputum culture 19, and necropsy 1. Compared with the remaining cases, patients with CAP due to *P. aeruginosa* had more frequently chronic obstructive pulmonary disease (COPD) (70% vs. 28.5%; $p < 0.001$) and were more commonly classified into Pneumonia Severity Index high-risk class (group V) (48% vs. 16%; $p = 0.001$). They also had received more frequently prior corticosteroid (30% vs. 6%; $p = 0.001$) and antibiotic (44% vs. 17%; $p = 0.011$) therapy. Multivariate analysis identified COPD (OR = 6.83) and prior corticosteroid therapy (OR = 4.22) as independent risk factors. Patients with CAP due to *P. aeruginosa* presented more frequently with septic shock at entry (25% vs. 3.8%; $p = 0.001$) and multilobar pneumonia (50% vs. 25.4%; $p = 0.019$) than the remaining patients. They were also given more frequently an inappropriate initial empirical antibiotic therapy (68% vs. 9%; $p < 0.001$). Early (29% vs. 2%; $p < 0.001$) and overall (55% vs. 8.5%; $p < 0.001$) case-fatality rates were higher among patients with CAP due to *P. aeruginosa*.

Conclusions: CAP caused by *P. aeruginosa* is uncommon and occurs mainly in patients with COPD treated with corticosteroids. It frequently presents with shock and causes high case-fatality rates. The risk factors delineated in this study should be considered when selecting initial empirical antibiotic therapy for patients with CAP.

O412 Predictors of positive microbiology in community-acquired pneumonia subjects: results of a multivariate analysis

J. Garau for the MOTIV Study Group

Objective: In prospective aetiological studies, a microbial cause is not found in 30–65% of subjects with community-acquired pneumonia (CAP). The objective of this analysis was to identify predictors of microbiological documentation in a large CAP trial.

Methods: In a multinational, phase III study conducted in hospitalised subjects with CAP, causative organisms were identified at baseline by culture, urine antigen testing and/or blood serology. 318 of 733 subjects in the ITT population had microbiologically documented CAP. A retrospective logistic regression analysis was performed to determine significant predictors for microbiological documentation.
Results: Baseline explanatory variables tested were: age (<65 vs ⩾65 years), gender, history of cardiac disease, history of respiratory disease, concomitant cardiovascular treatment, concomitant respiratory treatment, PSI risk class (II and III vs IV and V), SAPS score (⩽12 vs >12), severe CAP (as per modified ATS criteria), CURB65 score (0–3 vs 4–5), smoking history, alcohol consumption (abstinent–light vs moderate–heavy), shock, assisted ventilation, ICU admission, temperature (<39 vs >39ºC), C reactive protein (CRP; <20 vs >20 mg/dL), white cell count (⩽12 vs >12 G/L), previous systemic antimicrobial therapy, failure on previous systemic antimicrobials, multi-lobar or bilateral involvement, duration of infection (⩽4 vs >4 days). The following variables were significant predictors in the univariate analysis: PSI risk class ($p = 0.0043$), severe CAP ($p = 0.0031$), SAPS ($p = 0.0360$), alcohol use ($p = 0.0543$), concomitant cardiovascular treatment ($p = 0.0294$) and CRP ($p \leqslant 0.0001$).
After adjusting for potentially confounding variables, PSI risk classes IV–V (OR 1.4; 95% CI: 1.02–1.98), severe CAP (OR 1.4; 95% CI: 1.01–1.94), moderate–heavy alcohol consumption (OR 1.9; 95% CI: 1.10–3.24) and CRP ⩾ 20 mg/dL (OR 2.27; 95% CI: 1.66–3.2) were significant predictors of microbiologically documented pneumonia in the logistic regression model.
Conclusion: This study found that PSI, severe CAP (by modified ATS criteria), moderate-heavy alcohol consumption and baseline CRP value were associated with a higher microbiology recovery rate.

O413 Clinical implications of chest radiographic resolution in hospitalised adults with severe community-acquired pneumonia

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Objective: In follow-up of treatment of community-acquired pneumonia (CAP), chest radiographs (CXR) are regularly obtained. However, little is known about time to resolution of CXR abnormalities and correlation with clinical findings. We studied the rate of CXR resolution, evaluated factors associated with delayed resolution and determined the influence of CXR deterioration on prognosis in patients (pts) with severe CAP.
Methods: Of 288 pts enrolled in a multicentre study with severe CAP, clinical and laboratory data were prospectively obtained and CXR were taken on admission, at day 7 and at day 28. Pts were followed for 28 days. At days 7 and 28, CXR resolution, defined as absence of CXR abnormalities related to infection (pulmonary infiltrates, pleural fluid or atelectasis), and CXR deterioration, defined as new or progressive CXR abnormalities, was determined. Clinical improvement defined as a respiratory rate <25/min, saturation >90%, $PaO_2 > 55$ mmHg, haemodynamic stability and normal mental state was evaluated at day 7 and clinical cure, defined as discharged in good health at day 28.
Results: All pts [mean age 69.7 years (±13.9); 248 (86%) PSI > 90] had pulmonary infiltrates on admission. Follow-up CXR was available for 227 pts (79%) at day 7 and for 195 (68%) at day 28. At day 7, 57 patients (25%) had complete CXR resolution whereas 127 (56%) had clinical improvement (mean difference 31; 95% CI: 25–37). At day 28, 103 patients (53%) had complete CXR resolution and 152 (78%) had clinical cure (mean difference 25; 95% CI: 19–31). In multivariate logistic regression analysis, delayed CXR resolution was independently associated with *Streptococcus pneumoniae* infection (Odds Ratio [OR] 1.9; 95% CI: 1.2–3.1), multilobar disease (OR 2.9; 95% CI: 1.3–6.4), dullness to percussion (OR 6.9; 95% CI: 1.5–32.7), high C-reactive protein (>200 mg/l) (OR 4.2; 95% CI: 1.8–9.8) and high respiratory rate (>25/min) on admission (OR 2.4; 95% CI: 1.1–5.5). CXR deterioration during follow-up was not correlated with delayed clinical cure or mortality ($P > 0.6$).
Conclusion: Complete CXR resolution of severe CAP occurred in approximately a quarter of pts at day 7 and in about half at day 28. Persistence or deterioration of CXR abnormalities were not correlated with a poor prognosis. Therefore, routine follow-up CXR seems not to be appropriate for patients that respond to therapy and CXR follow-up to exclude a non-infectious cause should not be performed within 4 weeks after initial diagnosis.

O414 Is empiric broad-spectrum therapy always necessary in severe community-acquired pneumonia?

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Objectives: UK guidelines recommend co-amoxiclav/macrolide or a second/third-generation cephalosporin/macrolide for severe (CURB65 ⩾ 3) community-acquired pneumonia (CAP). Observational studies suggest that adherence to guidelines and atypical pathogen cover results in better outcomes. There is concern about the ecological impact, however, of broad-spectrum agents. This study compared mortality in hospitalised patients who had received amoxicillin/macrolide (narrow spectrum, NS) with those who had received either co-amoxiclav/macrolide or a second/third-generation cephalosporin/macrolide (broad spectrum, BS).
Methods: A retrospective cohort study was performed using data prospectively collected for a quality improvement (QI) study (Barlow et al. Thorax 2006). Patients were included if they had CAP and been treated with one of the regimens defined above. Patients were excluded if their diagnosis had changed before discharge. Descriptive statistics were stratified by type of therapy. Comparisons of 30-day mortality were also stratified by CURB65 criteria. Univariate and multivariate logistic regression were used to identify independent risk factors for 30-day mortality.
Results: Of 503 patients included in the QI study, 14 were excluded because of a change in diagnosis. Of the remainder, 378 (77%) had received one of the three regimens (NS = 148; BS = 230 of which 165 co-amoxiclav/macrolide and 65 cephalosporin/macrolide). As expected, there were significant differences ($p \leqslant 0.05$) between the NS and BS cohorts (exposure to the QI intervention, oral route availability, penicillin allergy, ID/respiratory ward, ID/respiratory consultant, CURB65 criteria, oxygenation, CRP, temperature, and ITU admission). When the cohorts were stratified for severity, 30-day mortality in the CURB65 ⩾ 3 cohort was higher in the BS group (43% versus 22%; see table). Age, oral route availability, respiratory rate, serum urea, and bilateral/multi-lobar changes on CXR were found to be independent risk factors for death using multivariate logistic regression (NS versus BS, $p = 0.14$ with the trend favouring NS).

30-day mortality stratified by antibiotic regimen and disease severity

CURB65 score	Amoxicillin/ macrolide	Co-amoxiclav/ macrolide	Cephalosporin/ macrolide	χ^2	p-value
0 or 1	2/69 (3%)	0/41 (0%)	1/14 (7%)	2.4	0.3
2	7/47 (15%)	5/45 (11%)	4/17 (23.5%)	1.5	0.5
⩾3	6/27 (22%)	28/75 (37%)	18/33 (54.5%)	6.6	0.036

Conclusions: Although confounding could explain our findings, the results do not support the paradigm that all patients with severe CAP require BS therapy. Better methods of identifying patients with severe CAP who would genuinely benefit from BS therapy and/or a randomised controlled trial are required.

O415 Magnitude of bacteraemia predicts one-year mortality

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Objectives: All hospitals in our region use the BacT/Alert® blood culture (BC) system with a 3-bottle BC set for adults. We hypothesised that the magnitude of bacteraemia (i.e., number of positive bottles in the initial BC set) predicts one-year mortality.

Methods: In a population-based study we analysed all patients with monomicrobial bacteraemia in North Jutland County, Denmark, 1996–2004. Data from the County Bacteraemia Registry were linked to the Hospital Discharge Registry (comorbidity) and the Danish Civil Registration System (vital status, including date of emigration or death). Patients with a BC index of 1 (i.e., one positive bottle) were chosen as the reference group. We computed Kaplan–Meier curves and performed Cox regression analyses to estimate mortality rate ratios (MRRs) with 95% confidence intervals [CIs] 30 and 365 days after the initial BC sampling date, first in crude analyses, second in analyses adjusted for age, comorbidity, acquisition of infection (community, nosocomial, or healthcare-related), and incident or recurrent episode. In addition we stratified the analyses on acquisition of infection and pathogen group.
Results: A total of 6,955 patients had 8,152 episodes of monomicrobial bacteraemia, among which one-year follow-up was possible for 8,108 (99.5%). Of these, 2,539, 1,511 and 4,058 episodes had a BC index of 1, 2 and 3, respectively. In crude analyses, 30-day MRRs were 0.98 [0.85–1.14] and 1.20 [1.07–1.34] for BC indices 2 and 3, respectively, and similar MRRs were found after 1 year (0.99 [0.89–1.09] and 1.12 [1.04–1.21]). All estimates remained unchanged in the adjusted analyses. Results for community-acquired and healthcare-related bacteraemia episodes were consistent with the non-stratified results, whereas all MRRs for nosocomial infections were close to 1. A BC index of 3 had the strongest long-term prognostic impact in pneumococcal bacteraemia (n = 855) (adjusted MRR 1.60 [1.09–2.34]).
Conclusions: In patients with community-acquired or healthcare-related bacteraemia, high magnitude of bacteraemia (i.e., a BC index of 3) predicted increased 30-day as well as 365-day mortality.

O416 CURB65 may predict 30-day mortality in patients with bacteraemia

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Objective: Bacteraemia is an important cause of mortality in hospitalised patients. CURB65 has been validated as a predictor of mortality in community-acquired pneumonia (CAP) (Lim et al. Thorax 2003), but not in other causes of sepsis. The aim of this study was to assess the performance of CURB65 in patients with bacteraemia.
Methods: A retrospective cohort study was performed using data routinely collected in the delivery of the Hull Bacteraemia Service. As part of this service, patients with confirmed bacteraemia are seen at the bedside by an infectious diseases physician. A typed report, which includes physiological data, is then sent to the patient's physician. Patients were included if they had all CURB65 criteria recorded at the point of bacteraemia. 30-day mortality was established by hospital database and stratified by CURB65 score. A receiver operating curve (ROC) was produced and area under the curve and 95% confidence intervals (CI) calculated.
Results: Of 151 patient reports, 61 patients (62% male) had a full set of CURB65 criteria. 49% of patients were over 65 years old and 34% were being managed on a renal ward. The most common bacteria were: MSSA (41% of patients), MRSA (23%); various Gram-negatives (16%); thought to be significant coagulase-negative staphylococci (15%); Group A–G streptococci (10%); and enterococci (8%). The most common sources of bacteraemia were: central venous line (31% of patients); intravenous drug use (15%); urinary tract (13%); contamination (10%); and skin/soft tissue (8%). 9% of patients were receiving discordant therapy prior to review. Overall, 30-day mortality was 25%. Recrudescence within 90 days occurred in 5.5% of patients. The table shows 30-day mortality stratified by CURB65 score. The area under the ROC was 0.73 (95% CI: 0.6–0.86).
Conclusions: This is the first study to assess the performance of CURB65 in a non-respiratory infection. Although preliminary, CURB65 appeared to stratify 30-day mortality in patients with bacteraemia. The cut-off for severe illness may need to be lower, however, than in CAP. There is the potential, therefore, to identify a low-risk cohort of patients (i.e. CURB65 = 0) who may be appropriate for an early switch to oral therapy and discharge from hospital. Likewise, patients at high risk (i.e. CURB65 $\geqslant$ 2) are more likely to need aggressive therapy according to the principals of the surviving sepsis campaign. A large prospective study is warranted.

30-day mortality stratified by CURB65 score in patients with bacteraemia

CURB65 score	30-day mortality	
	N	%
0	0/6	0
1	2/16	11
2	7/22	32
3	5/10	50
4	1/3	33
5	NIL	

O417 Risk factors and outcomes for persistent bacteraemia caused by *Staphylococcus aureus*

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Objectives: to assess risk factors and outcomes for persistent bacteraemia caused by *Staphylococcus aureus* (SA).
Methods: Clinically significant episodes of SA bacteraemia were registered prospectively. Medical records were reviewed; pertinent demographic, bacteriologic and clinical data were collected and the presumptive focus of infection was determined. Persistent bacteraemia was defined as positive blood cultures $\geqslant$48 hrs. Variables evaluated included: age, gender, functional capacity, healthcare-associated acquisition, co-morbid conditions, McCabe and Charlson scores, presence of prosthetic devices, intravascular and urinary catheters, mechanical ventilation, recent surgery or invasive procedures, steroid or cytotoxic therapy, neutropenia, source of infection, susceptibility to methicillin and the clinical status and laboratory findings at the time of the first positive blood culture.

Variable	No persistence	Persistent bacteraemia	P-value
Number of episodes	243	91	
Recurrent episode of SA bacteraemia	15 (6.2%)	14 (15.4%)	0.008
Pacemaker	3 (1.2%)	8 (8.8%)	0.01
Prosthetic joint	1 (0.7%)	4 (7.3%)	0.01
Source of infection			
Primary	85 (35.4%)	32 (36.0%)	
Catheter-related	14 (5.8%)	3 (3.4%)	
Skin, soft-tissues	50 (20.8%)	15 (16.9%)	
Joint, bone	8 (3.3%)	12 (13.5%)	
Pneumonia	22 (9.2%)	2 (2.2%)	
UTI	5 (2.1%)	0	
Endovascular	25 (10.4%)	14 (15.7%)	
Surgical site	20 (8.3%)	5 (5.6%)	
Other	11 (4.6%)	6 (6.7%)	

Results: We included 334 patients with SA bacteraemia. Statistically significant risk factors for persistent bacteraemia included a recurrent episode of SA bacteraemia, the presence of a pacemaker or a prosthetic joint, and endovascular, joint or bone infections as sources of infection (table). The difference for prosthetic valves was not significant (12.1% with vs. 9.9% without persistent bacteraemia, p = 0.56) and no

IVDUs were included in our cohort. Persistent bacteraemia was present among 48/197 (24.4%), 30/73 (41.1%) and 7/10 (70%) of patients who received appropriate antibiotic treatment, when appropriate treatment was started on days 0, 3 and 7, respectively. Echocardiography was performed for 70 (28.8%) patients without vs. 54 (59.3%) patients with persistent bacteraemia. Invasive procedures or interventions were performed more frequently with persistent bacteraemia (43/243, 17.7%, vs. 28/91, 30.8%, $p = 0.009$). No significant differences were noted with regard to complications at follow-up, including septic shock, mechanical ventilation, renal failure and days in ICU. Fever duration was longer with persistent bacteraemia (5.5 vs. 3.1 days, $p < 0.001$). All-cause 30-day mortality among patients alive at 48 h was 82/215 (38.1%) vs. 27/87 (31%), $p = 0.24$.

Conclusions: Identification of risk factors for persistent bacteraemia can support the performance of repeat blood cultures and direction of appropriate antibiotic treatment. With adequate management of foreign bodies, persistent bacteraemia may not portend a poorer prognosis.

O418 Clinical and microbiological features of 55 cases of staphylococcal toxic shock syndrome in France

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Objectives: To describe and compare the clinical presentation, the management, the outcome and microbiological features of menstrual and non-menstrual staphylococcal toxic shock syndrome (STSS).

Methods: We have prospectively collected cases of suspected STSS in France from December 2003 to June 2006. STSS was defined by the CDC diagnostic criteria and cases were divided in confirmed or probable TSS. The superantigenic toxin gene content were determinated by PCR for the corresponding isolates.

Results: Among 100 patients suspected to have staphylococcal STSS in the study period, 55 fulfilled the case definition of confirmed (20 cases) or probable STSS (35 cases), and 21 women (38%) presented with menstrual STSS versus 34 patients with non-menstrual STSS (62%). Non-menstrual STSS occurred in post-operative period in 7/34 cases (21%). Blood cultures were positive with *S. aureus* in 17 cases of non-menstrual STSS (50%) and never in those of MTSS ($p < 0.001$). The tst gene encoding TSST-1 was detected in all but one menstrual STSS cases (20/21, 95%). At least one gene encoding a superantigenic toxin was positive for all isolates responsible for non-menstrual STSS. Most of patients with non-menstrual STSS have primary focal infections, mainly localised on skin or mucous membranes (22/34 cases, 65%). Mortality occurred rapidly after the onset of symptoms (median 3 days; range 1–17 days) in seven patients with non-menstrual STSS (7/32, 22%). Deceased patients were more aggressively treated in comparison to survivors but they never received clindamycin, linezolid or intravenous immunoglobulins (IVIg).

Conclusion: We observed that non-menstrual STSS was a more severe disease as expected. Comparing with menstrual STSS, non-menstrual STSS patients have a high positive blood culture and mortality rate (50% and 22%, respectively). This could reflect an evolution of the *S. aureus* isolates towards a higher virulence and expression of various superantigenic toxins. Bacteraemia could partially explain the severity of non-menstrual cases and may be a composite form of shock, frontier between septic shock and STSS, potentially more severe than STSS itself. Specific therapeutic intervention has to be implemented for patients with non-menstrual STSS and we proposed the following strategy: rapid wound debridement whatever the importance of local inflammation; rapid addition of anti-toxinic antibiotic such as clindamycin or linezolid; and prescription of IVIg.

O419 Management of community-acquired bacterial meningitis

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Objectives: The purpose of the study was to evaluate the quality of the initial diagnostics and to identify clinical factors correlating to an unfavourable outcome. Hypothetically, sequelae and mortality could correlate to age, presence of risk factors, bacterial aetiology, and the time from the arrival to the hospital to the administration of relevant antibiotics (time-arrival–ab). The time-arrival–ab was thought to correlate with the quality of the initial diagnostics.

Methods: All culture positive CSF in Denmark from 2002 and 2003 were identified by the regional departments of clinical microbiology. Clinical data was collected retrospectively from medical charts from all bacterial meningitis (BM) patients in East Denmark.

Unfavourable outcome was identified as death or invalidating sequelae (e.g. total deafness). Positive outcome was identified as none, reversible or milder lasting means (e.g. deafness on one ear).

Results: 187 patients were included. 20% of patients admitted with the tentative diagnosis BM and 27% of patients with a typical clinical presentation got antibiotics later than 2 hours.

The mortality was 12% for patients receiving relevant antibiotics within 2 hours compared to 40% when antibiotics were administered later than 2 hours ($P = 0.00001$). The general mortality was 23%.

Age >50, presence of 1 or more risk factors, and non-meningococcal disease were also correlated with a unfavourable outcome ($p < 0.001$).

58% had a time-arrival–ab <2 hours. 73% of patients with a classical clinical presentation were treated within 2 hours compared to 29% with less typical presentation ($P < 0.001$).

Age was not correlated to classical clinical presentation.

By multivariate analysis age >50, late administration of antibiotics, non-meningococcal disease, and presence of risk factors were independently correlated with unfavourable outcome: Age >50 (OR 3.9, $P = 0.003$), non-meningococcal disease (OR 3.9, $P = 0.046$), presence of 1 or more risk factors (OR 2.4, $P = 0.018$), time-arrival–ab >2 hours (OR 1.6, $P = 0.007$).

Conclusion: A general correlation between time-arrival–ab and the severity of outcome was found. Age, non-meningococcal disease, presence of risk factors and time-arrival–ab >2 hours were independently correlated with unfavourable outcome in the multivariate analysis. The results may indicate that the outcome could improve further by optimal initial management of the BM patient.

Applied pharmacokinetics improve antimicrobial usage

O420 Penetration of telavancin into pulmonary epithelial lining fluid and alveolar macrophages

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Objectives: Telavancin (TLV), a novel, rapidly bactericidal lipoglycopeptide with a multifunctional mechanism of action against methicillin-resistant *Staphylococcus aureus* and other Gram-positive pathogens, is undergoing Phase 3 trials for hospital-acquired pneumonia. This study evaluated the level of TLV in plasma, and its penetration into pulmonary epithelial lining fluid (ELF) and alveolar macrophages (AM) after IV administration to healthy subjects.

Methods: Three daily doses were administered as 60-min TLV 10 mg/kg IV infusions to healthy subjects ($n = 20$; mean±SD age, 30±6 years). TLV was assayed (using liquid chromatography–tandem mass spectrometry) in blood taken preinfusion on Days 1 and 3, and at 0, 1, 3, 5, 7, 11 and 23 h after the Day 3 infusion, and in bronchoscopy samples obtained on Day 3 at 4, 8, 12 or 24 h after infusion initiation. Predictive modelling software simulated the pharmacokinetic disposition of TLV in different compartments.

Results: The pharmacokinetic model included plasma and peripheral compartments. Pulmonary ELF and AM appeared to be deeper extensions of the peripheral compartment. A chain of catenary compartments was incorporated to account for the delay in peak AM concentration. Mean (coefficient of variation [CV]) TLV intercompartmental clearances were 0.805 (9.2%), 0.000309 (49.1%), 0.00431 (38.4%), 0.334 (46.6%) and 0.247 (32.6%) mL/h/kg for CL21, CL23, CL24, CL32 and CL42,

respectively. Mean (CV) rate constants, k0 and k12, were 0.213 (5.6%) and 0.839 (17.4%) per h, respectively, and the mean (CV) volume of the central compartment was 62.2 (5.7%) mL/kg. The model predicted rapid equilibrium with plasma, slow transfer (CL23) to ELF and even slower transfer (delay + CL24) to AM. The maximum TLV concentration (Cmax) in ELF was ~3.7 μg/mL, which occurred 8 h post dose (Tmax) due to a very low clearance-in from the peripheral compartment. The TLV concentration profile of ELF was relatively flat, mainly because of the high clearance-out to the peripheral compartment. Cmax in AM (~50 μg/mL) occurred approximately 16 h after dosing. These simulations showed that TLV in ELF and AM have very different time–concentration profiles from those observed in plasma, where Tmax occurred 1 h after dosing and Cmax was 116 μg/mL.

Conclusion: Compartmental modelling results support the hypothesis that AM containing TLV originate in the circulation and that TLV penetrates deeply into non-infected pulmonary tissue.

O421 Pharmacodynamic evaluation of 7 antimicrobials recommended for secondary peritonitis, including the novel agent tigecycline, using global resistance data

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Objectives: Inappropriate empiric antibiotic therapy for secondary peritonitis (SP) promotes poor patient outcomes. The emergence of resistance in target organisms contributes to this issue. Tigecycline (TG) is a novel compound with activity against vancomycin-resistant *Enterococcus* (VRE) and methicillin-resistant *Staphylococcus aureus* (MRSA); it is newly indicated for intra-abdominal infections, and was equivalent to imipenem (IMP) in clinical trials. We predicted microbiological success against aerobic species causing SP by evaluating TG and 6 antimicrobials from classes recommended for SP by the Infectious Disease Society of America.

Methods: Cumulative fraction of response (CFR) via a 5000-subject Monte Carlo simulation was predicted for cefepime (FEP), ceftazidime (CAZ), ceftriaxone (CRO), IMP, levofloxacin (LVX), piperacillin-tazobactam (TZP) and TG using a pharmacodynamic analysis incorporating pharmacokinetic variability in patients and global minimum inhibitory concentration data. Excluding anaerobic bacteria, isolates collected in the 2004–2005 SENTRY and TEST Programmes were analysed by individual species then weighted by prevalence in SP, both with and without Enterococcus. A predicted CFR of 90% was considered desirable.

Results: Agents with predicted CFRs $\geqslant$90% when modeled against all species excluding *Enterococcus* were (dose, %CFR): FEP (1 g & 2 g q12h, 92 & 95); CAZ (2 g q8h, 94); IMP (500 mg q6h, 97); and TZP (3.375 g q6h, 91). TG displayed %CFR of 85 at the indicated dose of 50 mg q12h. CRO at 1 g and 2 g q24h had %CFRs of 76 and 78; LVX 750 mg q24h showed %CFR of 76. When *Enterococcus* was included in the analysis, IMP, TZP and TG showed %CFRs of 93, 88 and 87, respectively. Against individual species, TG had the highest %CFR vs. *Enterococcus*, at 100. Against *Escherichia coli*, the most prevalent aerobic species causing SP, all agents had $\geqslant$90%CFR except LVX (%CFR of 75).

Conclusions: Decisions regarding empiric therapy for SP should consider local epidemiology, as success is strongly influenced by species prevalence and susceptibility. Based on this analysis, resistance contributed to poor attainment of target exposures for CRO and LVX. When *Enterococcus* is not included, FEP 1 g or 2 g q12h or CAZ 2 g q8h given as combination therapy with an agent to cover anaerobes would be viable choices, as would monotherapy with IMP 500 mg q6h or TZP 3.375 g q6h. When *Enterococcus* is included in the epidemiologic mix, IMP, TZP and TG all appear to be viable monotherapeutic choices.

O422 Concentration-dependent selection of resistant mutants of *Enterococcus faecium* exposed to linezolid in an in vitro dynamic model

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Objective: Enterococcal resistance to linezolid (LZD) has been reported from a few clinical studies. Without pharmacokinetic data, these observations cannot be related to LZD concentrations with respect to placement in or out of the mutant selection window (MSW), i.e., the concentration range from the MIC to the mutant prevention concentration (MPC). To test the MSW hypothesis, *E. faecium* was exposed to LZD at concentrations that fell into the MSW for varying portions of the dosing interval (TMSWs).

Methods: A clinical isolate of *E. faecium* 392 (MIC 1.8 mg/L, MPC 7 mg/L) at a starting inoculum of 8 log CFU/mL was exposed to b.i.d. LZD for three consecutive days in a hollow-fiber two-compartment model. The simulated ratios of 24-hour area under the curve (AUC) to MIC were designed to provide TMSWs from 0 to 100 per cent of the dosing interval. Peripheral compartments of the dynamic model were sampled during and after treatment to determine bacterial counts and LZD concentrations (bioassay). Changes in *E. faecium* susceptibility and bacterial growth on agar plates containing 2×, 4×, 8× and 16×MIC of LZD were examined daily.

Results: The AUC/MICs were estimated at 33, 53 and 114 h, the respective steady-state peak concentration-to-MIC ratios were 4.7, 7.5 and 15.8 and TMSWs were 88%, 55% and 0%. At the two lower AUC/MIC ratios, *E. faecium* resistant to 2–16×MIC and 2–8×MIC of LZD, respectively, were selectively enriched with a concomitant slight loss in susceptibility. Neither growth on LZD-containing media nor changes in susceptibility occurred at the high AUC/MIC ratio that is comparable to the clinically attainable AUC/MIC for this strain (186/1.8 = 100 h).

Conclusions: This study suggests that (1) selection of mutants of *E. faecium* resistant to LZD depends on drug concentration; (2) both bacterial growth on LZD-containing plates and loss in susceptibility of *E. faecium* occur at AUC/MICs similar to those reported in earlier in vitro studies with fluoroquinolone-exposed staphylococci and pneumococci; (3) the MSW concept may be used to predict the anti-mutant concentrations of LZD.

O423 Pharmacodynamics of gentamicin against *Pseudomonas aeruginosa*: modelling bacterial response to drug-selective pressures

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Objective: We have previously developed a mathematical model predicting the response of *P. aeruginosa* (PA) to various fluctuating pharmacokinetic profiles of meropenem (Tam, ICAAC 05). However, the applicability of the model to other drug-pathogen combinations is unknown. We extended our model to predict PA response to fluctuating gentamicin exposures.

Methods: Time-kill studies with 10^7 cfu/mL of PA ATCC 27853 at baseline were performed. Gentamicin at 0, 0.5, 1, 2, 4, 8, 16×MIC was used for 24 h (MIC = 2 ug/mL). The experimental data were used to derive the best-fit estimates of the model parameters, and PA response to various gentamicin exposures over 72 h was predicted via computer simulation. Three parallel differential equations were used, each characterising the rate of change of drug concentration, overall susceptibility and microbial burden of the surviving bacterial population over time, respectively. Various gentamicin profiles ($t_{1/2}$ = 3 h) were investigated, corresponding to Cmax/MIC (dosing frequency) of 4 (q8), 12 (q24), 36 (q24) and 30 (q12). The computer simulations were subsequently validated using an in-vitro hollow fiber infection model (HFIM) with similar gentamicin exposures. Samples were obtained at baseline, 4, 8, 24, 48 and 72 hours to determine the bacterial burden.

Results: Time-kill studies data were satisfactorily captured by the model ($r^2 = 0.96$). A significant initial reduction in bacterial burden was predicted for all gentamicin exposures examined. However, regrowth over time due to resistance emergence was predicted for regimens with Cmax/MIC (dosing frequency) of 4 (q8), 12 (q24), and 36 (q24). Sustained suppression of bacterial population over 72 h was predicted with Cmax/MIC of 30 (q12). These predictions correlated well with our experimental data in HFIM.
Conclusions: The model was reasonable in predicting extended PA response to various fluctuating gentamicin exposures qualitatively, based on limited input data from time-kill studies. In view of its robustness and efficiency, our mathematical modeling and simulation approach holds great promise as a high-throughput screening tool for dosing regimen selection in antimicrobial (pre)-clinical development.

O424 PK/PD modelling of linezolid against MRSA

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Objective: In vitro PK/PD models, based on time-kill curve data, have become a powerful tool to predict the in vivo situation. To date, several physiology-based or semi-mechanistic modeling approaches have been undertaken to develop suitable PK/PD models that fit in vitro data sufficiently well. Widely used simple sigmoid Emax-models meet these criteria only partly. A further approach was undertaken to address the weak points of currently used models and applied to model the effects of linezolid against methicillin-resistant *Staphylococcus aureus* (MRSA).
Methods: Constant concentration time-kill curves were performed in triplicates in Mueller-Hinton broth (MHB, DIFCO) using linezolid concentrations, ranging from 0.25MIC to 16MIC. The change in number of viable bacteria (CFU/mL) versus time was linked to the antimicrobial effect of linezolid. MRSA OC 2878 was employed as the test organism. Samples were taken at 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 hours. A modified sigmoid Emax-model was fitted simultaneously to the data using the software Scientist® 3.0 for Windows™. To determine the best model, Model Selection Criterion (MSC) and coefficient of determination (R2) were taken into account as well as a visual inspection for goodness of fit.
Results: Two susceptibility stages were defined for MRSA – stage 1: self-replicating and susceptible to linezolid, and stage 2: unsusceptible and metabolic inactive. In the log-growth phase, susceptible bacteria grow with a growth rate constant k_s that is greater than their natural death rate constant k_d in a certain ratio. Converging to the stationary phase this ratio changes in a non-linear manner, described by an additional N_{max} term. In the presence of linezolid a concentration C dependent kill has to be taken into account. From certain drug specific concentrations on, a maximum effect is reached, described by the maximum kill rate constant k_{max}. However, both the onset of growth and kill can be delayed and modeled by exponential terms, characterised by time t and dg or dk, respectively. The final shape of the curve is smoothed out by a Hill factor/shape factor h. The modified sigmoid Emax-model shown below described the data best, resulting in MSC and R2 values of 4.04 and 0.82.

$$\frac{dN}{dt} = \left\{ k_s \cdot \left(\frac{k_s \cdot \left(1 - \frac{N}{N_{max}}\right) + k_d}{k_s + k_d} \right) \cdot (1 - e^{-dgt}) - \left[k_d \cdot \left(\frac{k_s \cdot \left(1 - \frac{N}{N_{max}}\right) + k_d}{k_s + k_d} \right) \cdot \left(1 + \frac{k_{max} \cdot C^h}{EC_{50}^h + C^h} \right) \right] \cdot (1 - e^{-dkt}) - k_d \cdot \left(1 - \frac{k_s \cdot \left(1 - \frac{N}{N_{max}}\right) + k_d}{k_s + k_d} \right) \right\} \cdot N.$$

Conclusion: The proposed model incorporating five additional terms could account much better for the in vitro situation than a simple Emax-model. A simultaneous fit describes the actual time-kill curve data sufficiently well.

O425 Comparative pharmacodynamics of telavancin and vancomycin with *Staphylococcus aureus*: multiple-dose simulations using an in vitro dynamic model

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Objective: The anti-staphylococcal pharmacodynamics and killing kinetics of telavancin (TLV) and vancomycin (VAN) against methicillin-resistant *S. aureus* ATCC 43300 were studied in five-day treatment courses over a wide range of ratios of the 24-h area under the curve (AUC) to MIC.
Methods: MICs of TLV and VAN for *S. aureus* ATCC 43300 were 0.25 and 0.8 mg/L, respectively. The respective mutant prevention concentrations (MPCs) were 5 and 18 mg/L. An 18-hour culture of *S. aureus* ATCC 43300 at a starting inoculum of 8 log CFU/mL was exposed to mono-exponential concentration decays of TLV (once-daily dosing, half-life of 8 h) and VAN (twice-daily dosing, half-life of 6 h). With each antibiotic, peak concentrations were designed to be equal to the MIC, above the MIC but below the MPC, i.e., within the mutant selection window (MSW), and above the MPC. The respective steady-state AUC/MICs of TLV varied from 50 to 1200 h and those of VAN varied from 30 to 1200 h. To determine the antimicrobial effect, the area between the level corresponding to the starting inoculum and the time-kill curve (ABBC) was calculated from time zero to 144 h. To detect possible changes in susceptibility to TLV or VAN, bacterial growth on agar plates containing 2×MIC and 4×MIC of antibiotic was recorded and the MICs were tested every 24 h.
Results: With both TLV and VAN, anti-staphylococcal effects depended on the AUC/MIC ratio: the higher the AUC/MIC, the greater the killing and delay of bacterial regrowth. Linear relationships of ABBC to log AUC/MIC were established with each antibiotic. Based on these relationships, the anti-staphylococcal effect of the proposed therapeutic dose of TLV (10 mg/kg; AUC/MIC 3400 h) is 25% greater than that of two 1-g doses of VAN given at a 12-h interval (AUC/MIC 500 h). Regardless of whether TLV and VAN concentrations were in or out of the MSW, no bacterial growth occurred on antibiotic-containing plates and no change in susceptibility was observed.
Conclusion: These findings suggest greater anti-staphylococcal efficacy of TLV relative to VAN at their clinically achievable AUC/MICs.

O426 In vivo pharmacokinetics and intracellular accumulation of five antistaphylococcal agents in a murine model of peritonitis

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We have recently evaluated a number of antistaphylococcal drugs in vivo in a murine model of peritonitis, with respect to their intracellular antistaphylococcal activity.
Objectives: (1) A PK description of azithromycin (AZM), cefuroxime (CXM), dicloxacillin (DCX), gentamicin (GEN) and rifampicin (RIF) in plasma and peritoneal exudate following SC injection. (2) A description of the intracellular accumulation of antibiotics in peritoneal exudate.
Methods: Female NMRI mice were inoculated IP with *Staphylococcus aureus* E19977 in 5% mucin. Two hours later, mice were treated SC with one of the five antibiotics. For each individual mouse an aliquot of peritoneal exudate was saved for differential somatic cell count and measurement of antibiotic concentration. Pelleted cells (300 g, 10 min) from the rest of the peritoneal exudate were lysed in a small volume of dH_2O. Antibiotic concentrations were measured by bioassay. Intracellular accumulation was calculated by dividing the intracellular with the extracellular concentration.
Results: Cmax (total drug) in plasma and peritoneal exudate, respectively, was 4.4 and 0.6 mg/L (AZM), 370 and 172 mg/L (CXM), 375 and 175 mg/L (DCX), 19 and 12 mg/L (GEN) and 13 and 5.3 mg/L (RIF). T > MIC (total drug) in plasma and peritoneal exudate, respectively, was 42 and 12 min (AZM), 123 and 209 min (CXM), 273 and 273 min (DCX), 132 and 216 min (GEN) and 267 and 267 min (RIF). Intracellular

accumulation (range) of antibiotics (total drug) found was 26–106 (AZM), 4–19 (CXM), 2–13 (DCX), 21–36 (GEN), 2–25 (RIF).
Conclusion: The concentrations found for CXM and DCX were higher than the concentrations found for AZM, GEN and RIF. This corresponds well with the higher dose injected and the relatively high antistaphylococcal activity observed in PD studies. The intracellular accumulation of AZM and RIF found here corresponds well with what has previously been reported. The intracellular accumulation for CXM, DCX and GEN found here, are surprisingly high, since β-lactams are notoriously considered not to accumulate intracellularly, and the 2–4 fold intracellular accumulation of aminoglycosides take several days, but the influx correlates well with the good effect seen in the in vivo model.

O427 Proinflammatory effects of erythromycin, clarithromycin and azithromycin on human endothelial cells in vitro

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An inflammatory reaction of the venous vessel is a common clinical problem that is observed after intravenous application of antibiotics and other drugs. The local irritation of the endothelium at the site of infusion leads to an inflammatory response with an increased expression of various cell surface antigens. Among these are CD 34, E-Selectin (CD 62E), ICAM-1 (CD 54) and VCAM-1 (CD 106). We studied the effects of three closely related antibiotics on human endothelial cells in vitro. We used the endothelial cell line EA.hy 926 and analysed the reaction by means of flow cytometry (FACScan, Becton Dickinson). Cells were incubated with clarithromycin or azithromycin at concentrations ranging from 100 mg/l to 800 mg/l and at concentrations ranging from 200 mg/l to 1400 mg/l for erythromycin. Such concentrations occur under therapeutic conditions at the site of infusion. Subsequently, they were stained with fluoresceinisothiocyanate- or phycoerythrin-conjugated monoclonal IgG mouse-antibodies for the four antigens mentioned above. Cells were incubated with the drugs for 2 h and analysis was carried out after an additional time period of 22 h. In control cells, we found positively stained cells at the following levels: CD 34 (2%), E-Selectin (4%), ICAM-1 (14%) and VCAM-1 (2%). The most pronounced changes were observed at 800 mg/l (erythromycin), 600 mg/l (azithroymcin), and 400 mg/l (clarithromycin). Erythromycin (800 mg/l) caused significantly increased expressions of all epitopes [CD 34 (+6%), E-Selectin (+5%), ICAM-1 (+15%) and VCAM-1 (+5%)]. At 600 mg/l the azalide azithromycin provokes a stronger upregulation of the proinflammatory antigens: CD 34 (+17%), E-Selectin (+16%), ICAM-1 (+27%) and VCAM-1 (+17%). Clarithromycin at a concentration of 400 mg/l causes a similar effect as erythromycin at twice this concentration [CD 34 (+6%), E-Selectin (+7%), ICAM-1 (+23%) and VCAM-1 (+4%)]. Analysis of the cell surface markers involved in cell-cell-interactions proved to be a useful approach to further study the mechanism of infusion phlebitis and to compare the proinflammatory effects of related compounds in vitro.

O428 Aminoglycoside-induced apoptosis in cultured renal (LLC-PK1) and non-renal (J774 macrophages) cells: comparison between gentamicin and amikacin

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Objectives: Apoptosis is now recognized as an early, and probably critical determinant in gentamicin (GEN)-induced nephrotoxicity in animals (Antimicrob Agents Chemother. 2000; 44: 665–75) as well as in renal cultured cells (Toxicol Sci. 2000; 56: 229–39). Models using electroporated cells also show that direct delivery of GEN in the cytosol of cultured renal cells enhances at least 30-fold its capacity to induce apoptosis (Antimicrob Agents Chemother. 2006; 50: 1213–21). Our aims were (i) to examine whether the capacity of GEN to induce apoptosis is restricted to renal cells; (ii) to compare amikacin (AMK) to GEN in this context, since AMK is generally considered to be less nephrotoxic than GEN (Antimicrob Agents Chemother. 1999; 43: 1003–12).
Methods: We used non-confluent murine J774 macrophages and porcine LLC-PK1 renal cells grown to 80% of confluency. Electroporation was performed on trypsinised LLC-PK1 cells (8 square wave pulses; 800 v/cm; 1 ms) as previously described (Antimicrob Agents Chemother. 2006;50:1213–21). Cell viability was checked by measurement of LDH release (only cultures with <10% release were used for evaluation). Apoptotic cells were enumerated after DAPI staining by observers unaware of the experimental conditions, and expressed as percentage of all visible cells.
Results: The Table shows the extent of apoptosis observed in controls (no aminoglycoside), in cells exposed to GEN concentrations known from previous studies to induce marked apoptosis or to AMK equimolar concentrations. For GEN, apoptosis developed on a concentration-dependent manner from an extracellular concentration of 1 mM for incubated cells and from 32 μM for electroporated cells. For AMK, no significant increase of apoptosis was seen at concentrations tested.

	% apoptotic cells					
	J774 macrophages incubated[a]		renal LLC-PK1 cells incubated[b]		electroporated[c]	
	none	3 mM	none	3 mM	none	128 μM
Gentamicin	0.1	14.9	1.5	12.7	1.8	18.4
Amikacin	0.0	1.2	1.5	0.0	0.8	0.9

[a]24 h incubation; [b]48 h incubation; [c]24 h incubation in drug-free medium after electroporation in the presence of the drug.

Conclusions: Apoptosis develops in both renal and non-renal cells upon incubation with GEN. The lack of apoptosis observed with AMK with both incubated (renal and non-renal) and electroporated (renal) cells support the concept that this aminoglycoside is intrinsically less toxic than GEN.

O429 Inhibitors and activator of the P-glycoprotein (P gp) efflux pump modulate the accumulation of daptomycin (DAP) in THP-1 macrophages and its intracellular activity towards *Staphylococcus aureus*

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Objectives: The concentration of antibiotics in eukaryotic cells can be reduced by efflux pumps such as P-gp, which may impair their activity against intracellular bacteria (JAC 2003; 51: 1167–1173). DAP is a lipopeptide antibiotic with an amphiphilic character (calculated logD at pH 7 and 25ºC: 9.56). Since amphiphilicity is an important determinant for recognition and transport by P-gp, we have examined whether the activity of this transporter in macrophages could affect the handling and intracellular activity of DAP.
Methods: Uninfected THP-1 macrophages were exposed to DAP (24 h) or to the fluorochrome dimethyloxadicarbocyanine iodide (DIOC2; a specific P-gp subtrate; 5 h) and their cell contents measured by fluorimetry (see JID 2005; 191: 2149–2152 for assay method of daptomycin). Intracellular activity of DAP was measured against phagocytised *S. aureus* (ATCC 25923; MIC in Ca^{2+} supplemented MH broth: 0.125 mg/L) after a 24 h exposure to an extracellular concentration of DAP yielding an apparent static effect for intracellular bacteria (1 mg/L; see AAC 2006; 50: 841–851 for description and validation of the model with other antibiotics). The activity of P-gp was inhibited by incubation with verapamil (100 μM) and GF120918 (0.25 mg/L), or stimulated with ouabain (1 mM). Gemfibrozil (an inhibitor of the MRP transporters) was used as control.
Results: The data presented in the Table show that P-gp modulators affect the cell handling and the intracellular activity of DAP in the same directions (verapamil and GF120918 increase DAP accumulation and activity, while oubain decrease them both), whereas a MRP inhibitor is without effect.

Condition	Cellular accumulation[a]		DAP intracell. activity
	$DIOC_2$	DAP	Δ log cfu[b]
Controls	547±39	0.29±0.1	0.0±0.1
+ verapamil	726±25*	0.68±0.1*	−1.4±0.1*
+ GFl 20918	Not possible[c]		−1.3±0. 1*
+ ouabain	106±29*	0.10±0.0*	+ 0.4±0.1*
+ gemfibrozil	485±36[ns]	0. 25±0.1[ns]	+0.1±0.1[ns]

[a]Apparent cellular to extracellular concentration ratio.
[b]Change in cfu at 24 h compared to post-phagocytosis inoculum (~10^6 cfu/mg cell protein).
[c]No assay possible because of interference with GFl 20918 intrinsic fluorescence.
*Significantly different from control ($p < 0.05$).
[ns]Not significantly different from control.

Conclusions: DAP is a substrate of the P-gp efflux transporter in human THP-1 macrophages, which reduces its accumulation and intracellular activity.

Epidemiology of extended-spectrum β-lactamase/metallo-β-lactamase

O430 Detection of CTX-M-14 β-lactamase in *Escherichia coli* from a long-term care and rehabilitation facility in northern Italy

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Objectives: The presence of ESBL producers (particularly among uropathogens) in geriatric long-term care and rehabilitation facilities (LTCRFs) has not been investigated in Italy, until now. The objective of this study was to evaluate the diffusion of CTX-M type β-lactamases among *Escherichia coli* clinical isolates from LTCRFs.
Methods: During the period April 2003–May 2004, a total of 529 *E. coli* consecutive non-replicated isolates were collected from inpatients at the LTCRF-RSA ASP "Golgi-Redaelli" of Milan. All isolates were identified and screened for ESBL production using the GNS-530 card of the VITEK 1 system, the double-disk synergy test and/or the confirmatory CLSI method. IEF coupled with a bioassay, PCR amplification and sequencing were carried out to identify the nature of the blaCTX-M-type determinants. Conjugation experiments, plasmidic DNA extraction followed by restriction analisys were also performed. The clonal relationships between the isolates were evaluated by PFGE of genomic DNA digested with NotI.
Results: All the *E. coli* clinical isolates resulted susceptible to piperacillin-tazobactam; 69/529 (13%) were ESBL producers. Such strains were obtained mainly from urinary samples (92.8%), in most cases from catheterised patients (90.6%). The CTX-M-type enzymes detected in 52/69 (75.4%) *E. coli* isolates showed a pI 8.2 or 8.4 active on cefotaxime, cefepime, and aztreonam. 40/52 (76.9%) CTX-M positive isolates were found to produce also a TEM-1 enzyme. The CTX-M determinants were transferable in 20/52 (38.4%) cases.
Sequencing results showed that 4/52 (7.7%) strains were CTX-M-14 producers while the remaining were CTX-M-1 producers. There was a common plasmid in 2/4 of the CTX-M-14 producers, both strains have been isolated from the Golgi institute. After plasmids restriction analysis, the CTX-M-1 coding plasmids exhibited different restriction profiles, while 2/4 CTX-M-14 producers resulted clonally related. PFGE profiles demonstrated the presence of at least 9 different clones.
Conclusions: Finding the different CTX-M-1-encoding plasmids in clonally related and unrelated strains of *E. coli* from the RSA ASP "Golgi-Redaelli" indicates a notable spreading potential for these plasmids and suggests that horizontal transfer could be the principal, but not the only mechanism of CTX-M enzymes spreading in the hospital environment. This is the first report of a CTX-M-14 gene in *E. coli* in Italy.

O431 Epidemiology and genetic features of CTX-M-15-producing *Klebsiella pneumoniae* epidemic clones in Hungary in 2005

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Objectives: To investigate the dissemination and molecular epidemiology of CTX-M-15-producing *K. pneumoniae* epidemic clones (KP-EC) identified earlier in Hungary by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST).
Methods: 396 ESBL-producing KP clinical isolates were submitted to the National ESBL Reference Laboratory for confirmation in 2005. On the basis of phage types 190 isolates from 38 healthcare facilities were selected for testing by PFGE and representative isolates were further characterised by MLST.
Antimicrobial susceptibility testing was performed by disk diffusion according to the CLSI. The carriage of blaCTX-M and ISEcp1 were investigated by PCR and CTX-M amplicons sequenced. The transferability of ESBL genes in representative isolates was tested and the plasmid profiles analysed.
Results: Three CTX-M-15-producing KP-ECs were identified all showing high level resistance to ciprofloxacin. The previously described *K. pneumoniae* Hungarian epidemic clone (HEC) spread to 31 healthcare facilities, affecting 124 patients, and causing 3 nosocomial outbreaks. This clone proved identical to sequence type (ST) 15, carried two gyrA mutations, a single parC mutation and blaSHV-28. The second epidemic clone spread to 4 healthcare facilities, affecting 45 patients and causing 2 nosocomial outbreaks. This clone represents a novel sequence type (data submitted to MLST centre), carrying a single gyrA and a single parC mutation. The third epidemic clone spread to 3 healthcare facilities, affecting 21 patients and causing one nosocomial outbreak. This clone corresponds to ST 11, carrying two gyrA and a single parC mutations. ISEcp1 was detected exclusively in strains belonging to the ST 11 clone.
Conclusion: In 2005 the number of infections caused by CTX-M-producing KP rose sharply in Hungary. In addition, a shift was demonstrated in the occurrence of CTX-M-producing KP clones. Three multidrug resistant KP-ECs were detected in 38 healthcare facilities causing large outbreaks and individual nosocomial infections.

O432 Polyclonal distribution of extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in Stockholm

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Objectives: Previously published Swedish reports on the epidemiology of extended-spectrum β-lactamase (ESBL)-producing Enterobactericaeae have identified CTX-M as the most prevalent ESBL-group in Sweden. However, no previous reports on the baseline epidemiological situation in the absence of outbreaks have been published from Sweden. This study aimed at genotypic characterisation of all ESBLs at the Karolinska University Hospital in Stockholm during the entire 2005.
Methods: The prevalence of ESBL-production among *E. coli* and *K. pneumoniae* at the Karolinska University Hospital was 0.7% in 2005. ESBL-positive clinical isolates (n = 160) of *E. coli* and *K. pneumoniae* collected at the Northern (n = 63) and Southern (n = 97) campus of the hospital during 2005 were investigated. Most isolates (59%) were derived from out-patient sources, and the majority were from the urinary tract (81%). Among the remaining 41% of the isolates, 15% were derived from nursing homes and the rest from hospital in-patients. PCR was performed with primers covering all known CTX-M variants, and specific primers for the CTX-M-1 and -9 groups. CTX-M-negative isolates were analysed with primers targeting TEM. Subsequently DNA sequencing was performed. Epidemiological typing was performed with the PhenePlate (PhP) system. MICs for ceftazidime, cefotaxime and cefpodoxime were determined with Etest, and susceptibility testing for other antibiotics was performed with disk-diffusion.

Results: Among isolates from Northern Stockholm, 59/63 isolates were positive for CTX-M. CTX-M-15 was the most prevalent subtype (39/59). CTX-M-14 was found in 17/59 isolates, while 7 isolates produced CTX-M-2-like or TEM-derived ESBLs. From Southern Stockholm 92/97 isolates were found to be CTX-M positive, out of which 72 were CTX-M-15 and 17 were CTX-M-14. Two isolates produced CTX-M-22, and the rest produced either CTX-M-2-like or TEM-derived ESBLs. The PhP-typing revealed the presence of one Northern clone (n = 16) and one Southern clone (n = 13). All isolates were resistant to cefpodoxime, and 70% were resistant to ciprofloxacin. In vitro susceptibility to ceftazidime (according to EUCAST) was observed in some CTX-M-14 isolates, and some TEM-producers were cefotaxime susceptible.
Conclusions: This study identified CTX-M-15 as the dominating ESBL genotype in Stockholm. Although two clones of respectively 14 and 16 isolates could be identified, the majority of the strains were clonally unrelated.

O433 Faecal carriage of extended-spectrum β-lactamase-producing *Escherichia coli*: prevalence and risk factors in different populations

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Objectives: Extended-spectrum β-lactamase-producing *Escherichia coli* (ESBLEC) is an emerging cause of community acquired infections. We investigated the prevalence of faecal carriage of ESBLEC in different groups of patients and the variables associated with carriage.
Methods: Faecal carriage of ESBLEC was investigated from June 2005 to September 2006 in 4 groups of persons: outpatients from whom ESBLEC had been isolated from a clinical sample (group A); living-together relatives (group B1); non-living-together relatives (group B2); and patients randomly chosen among those being attended at the emergency department (group C). Rectal swabs were obtained from all of them and inoculated in MacConkey-cefotaxime (2 mg) and MacConkey-ceftazidime (2 mg). ESBL production was studied following CLSI recommendations. The following data were collected: demographics, previous healthcare relation, co-morbidities, previous use of antimicrobials and proton-pump inhibitors, invasive procedures, feeding habits, and pets. Multivariate analysis of the association of the different variables with faecal carriage were performed using logistic regression.
Results: We included 54, 74, 32, and 54 patients in groups A, B1, B2 and C, respectively. Rectal colonisation by ESBLEC was detected in 36 (68%), 22 (30%), 5 (16%) and 4 (7%) persons from groups A, B1, B2 and C, respectively. The prevalence rates were significantly different between group A and all others ($p < 0.001$), and between groups B1 and C ($p = 0.02$). Among people in groups B1, B2 and C, multivariate analysis showed higher risk of colonisation in relatives of group A patients, either living with them (OR = 16.1; 95% CI: 4.1–63.6; $p < 0.001$) or not (OR = 6.2; 95% CI: 1.2–29.8; $p = 0.02$), and in those with co-morbidities (OR = 3.8; 95% CI: 1.4–10.2; $p = 0.008$), and lower risk in those eating out more frequently (OR = 0.3; 95% CI: 0.1–0.9; $p = 0.02$) and eating pork more frequently (OR = 0.6; 95% CI: 0.3–1.0; $p = 0.06$). Previous antimicrobial use was not associated with increased risk.
Conclusions: Faecal carriage of ESBLEC is more frequent in relatives of patients from which ESBLEC is isolated from a clinical sample than in non relatives, suggesting either person to person transmission of isolates/genetic elements, or acquisition from a common source. Some feeding habits were associated with carriage, but previous antimicrobial use was not. Molecular studies are progress to further elucidate the epidemiology of faecal carriage of ESBLEC.

O434 Occurrence of ESBL-producing *Escherichia coli* in outflow from a wastewater treatment plant

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Objectives: This study describes the isolation of extended spectrum β-lactamase (ESBL) producing *E. coli* strains in outflow from a municipal secondary wastewater treatment plant. The role the environment plays in the dissemination, maintenance and amplification of antimicrobial resistance is an area of increased scrutiny. Few studies have examined surface waters and effluent for the presence of antimicrobial resistant organisms.
Methods: Six samples (1 per week) were collected from the outflow of a municipal secondary wastewater treatment plant between August and September 2006. These were screened for the presence of *E. coli* resistant to ampicillin, cefoxitin, cefotaxime, and ciprofloxacin. Resistant isolates were tested for susceptibility to sixteen antimicrobial agents by Clinical Laboratory Standards Institute (CLSI) disk diffusion methods, including the following β-lactams: ampicillin, cefpodoxime, cefotaxime and ceftazidime. ESBL production was confirmed by the CLSI combination disk method for ESBL production using cefpodoxime. Confirmed ESBL producers were screened for blaTEM, blaSHV and blaCTX-M by PCR using specific primers. Relatedness of ESBL producers was determined by pulsed field gel electrophoresis (PFGE) using XbaI.
Results: Thirty-four isolates of *E. coli* resistant to one or more antimicrobial agents tested were identified. Seven (21%) were confirmed as ESBL producers. All 7 confirmed ESBL producers were resistant to 3 or more antimicrobial agents tested. PCR revealed a blaTEM gene in all 7, and a blaCTX-M group 1 gene in 3 isolates. PFGE analysis identified 5 pulsed field profiles (PFPs). These were isolated from samples taken on 3 separate dates. Isolates with indistinguishable PFPs were isolated from samples taken on different dates. Isolates with distinguishable PFPs were isolated on the same date.
Conclusion: This is the first report of the occurrence of ESBL-producing *E. coli* in the outflow from a secondary wastewater treatment plant and reflects the extent to which this resistance phenomenon is now very widely disseminated.

O435 Prevalence of ESBL in the Netherlands: the ONE study

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Background: The prevalence of extended spectrum β-lactamase (ESBL) producers is increasing worldwide. Data on prevalence in The Netherlands are sparse. Therapeutic options for ESBL producers are generally limited to fluoroquinolones, aminoglycosides and carbapenems. Ertapenem is a new broad spectrum carbapenem that may be suited to use against ESBL-producing enterobacteriaceae. Aim of the ONE study was to determine the prevalence of ESBL in the Dutch population and the susceptibility of ESBL producers against Ertapenem and other antibiotics.
Methods: 22 laboratories equally distributed in the Netherlands participated in the study. Each lab was asked to collect up to 100 consecutive enterobacteriacea isolates from clinical samples during a 4 month period. Isolates from rectal and nose swabs were excluded, as were isolates from surveillance cultures. A maximum of one isolate per species per patient was allowed. Isolates were identified by participating laboratories using their own standard identification technique. MICs were determined using Etest on site for ertapenem, amoxicillin, amoxicillin/clavulanic acid, meropenem, piperacillin/tazobactam, ceftazidime, ceftriaxone, tobramycin, cotrimoxazole and ciprofloxacin. Control ATCC strains were included. Afterwards, strains were collected by the central lab for further analysis. For ESBL screening, primary isolates of *Escherichia coli* and *Klebsiella pneumoniae* were subcultured on a ESBL screening plate with ceftazidime and cefotaxim. Colonies growing on the screening plate were tested for ESBL using the Etest TZ/TZL and CT/CTL strip and if necessary the PM/PML strip. *K. oxytoca* was evaluated based on Etest results of ceftazidime and ceftriaxon. All other enterobacteriacae were tested with the Etest PM/PML strip to differentiate the ESBL from the AmpC producers.
Results: The overall prevalence of confirmed ESBL producers was close to 6%. 43% of all isolates were identified as *E. coli* and 12% as *K. pneumoniae*, with ESBL prevalences of up to 6% and 8%, respectively. The MIC90's (mg/L) were 0.38 for ciprofloxacin, 0.125 for

ertapenem, 0.094 for meropenem, 32 for cotrimoxazole and 2 for tobramycin.
Conclusion: This was the first large scale survey to determine the prevalence of ESBL-producing enterobacteriaceae in the Netherlands. The overall prevalence of close to 6% is significant and indicates that there is reason to start monitoring ESBL prevalence on a regular basis in order to better guide control measures.

O436 Genetic characterisation of highly prevalent MBLs and OXA carbapenemases in multidrug-resistant *Acinetobacter baumannii* strains in a Romanian hospital

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Objective: This study was designed to demonstrate (1) the clonal relationship, (2) the prevalence and type of acquired metallo-β-lactamases (MBLs) and oxacillinases with carbapenem-hydrolyzing activity and (3) the potential risk of integron dissemination among multidrug-resistant *Acinetobacter baumannii* strains isolated from cardiovascular devices associated infections.
Material and Methods: Multiresistant *A. baumannii* isolates collected between 2003 and 2006 at the Institute for Cardiovascular Diseases C.C. Iliescu were tested by VITEK 2 automatic system, IPM disc Hodge test, IPM-EDTA+SMA double disk synergy tests, double disk test in MH agar plus cloxacillin (250 mg/l), microdilution tests of β-lactams and colistin, IEF, PCR for blaIPM-1, blaVIM-1, blaVIM-2, blaOXA-23, blaOXA-24, blaOXA-51 and blaOXA-58, class 1- and class-2 integrons and sequencing for MBL-, OXA-types carbapenemase-encoding genes and integrons. The clonal relationship between the isolates was evaluated by PFGE.
Results: 120 IPM-resistant (MIC 8–64 mg/l) *A. baumannii* isolates were MBL- and multiple OXA-types producers carrying blaIPM-1 and blaVIM-2 alleles, blaOXA-23 and blaOXA-58-plasmid mediated genes, respectively. PFGE showed six different clonal lineages. The blaVIM2 gene was carried on a gene cassette inserted into class-1 integrons, identical to those from *P. aeruginosa* in Europe and which included 5 different known additional cassettes (arr-3, catB3, aadA1, aacC1 and aacA4).
Conclusions: This study is the first report of the integron-associated IMP resistance, high prevalence and co-existence of blaIPM-1, blaVIM-2 and blaOXA-23-type among multiple clones of blaOXA-8-type-bearing multiresistant clinical isolates of *A. baumannii* in Romania. Taking into account the very narrow antibiotherapy choices in these infections, the horizontal transfer of these genes and also the increasing resistance to colistin, the possibility of a further spreading of carbapenem-hydrolyzing oxacillinases is of a considerable concern for antimicrobial chemotherapy.

O437 Diverse metallo-β-lactamases and integron gene cassette arrays in *Pseudomonas* spp. and *Enterobacter cloacae* isolates from Warsaw, Poland

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Objectives: To determine the mechanism of resistance and genetic structures containing resistance genes of 40 multiresistant clinical isolates collected in Warsaw, Poland from 2002–2005.
Methods: 31 *Pseudomonas aeruginosa* (PSA), 7 *P. putida* (PPU), 1 *P. stutzeri* (PST) and 1 *Enterobacter cloacae* (EC) isolates, positive by Etest MBL strips (ABbiodisc) were collected in the years 2001 (1), 2002 (3), 2003 (10), 2004 (11), and 2005 (15). The strains were investigated for the presence of metallo-β-lactamase genes by class-1 integron PCR followed by digestion with restriction enzymes, Southern hybridisation and probing with blaVIM probes. Positive isolates were further investigated by PCR and sequencing with primers designed against class-1 integron, blaVIM genes and Tn5090 transposase genes.
Results: All isolates were positive by hybridisation with blaVIM-1 and blaVIM-2 radio-labelled probes. Digestion of the class-1 integron PCR products with HincII produced ten different integron RFLP types (Types A–J). Type A was found in six PSA, 3 PPU and one PST strains and consisted of a class-1 integron harbouring aacA4 and blaVIM-4 gene cassettes. Type B was found in 10 PSA strains and the individual EC strain, harbouring an integron with aacC4, blaVIM-4 gene cassettes and the insertion sequence ISPpu17. Eight strains harboured the blaVIM-2 gene cassette in several different gene arrays and only one strain of PSA harboured the blaVIM-1 gene cassette. All class-1 integrons were of the more common form including the 3′Conserved sequence. No class-1 integrons were found that did not have the 3′CS as has been found in several recent blaVIM-2 isolates from diverse geographic regions.
Conclusion: Metallo-β-lactamases appear to be increasingly detected in clinical bacterial isolates from the Warsaw area of Poland, from the initial isolate in 2001 to fifteen isolates in 2005. The most commonly isolated MBL genes in this region are the blaVIM-4 fused gene cassette and the blaVIM-2 gene cassette. Other gene cassettes commonly found are aacC4, aadB and aadA10, conferring resistance to aminoglycosides. The finding of the same integron RFLP types in different species of bacteria indicates increasing horizontal dissemination of these broad-spectrum resistance determinants.

O438 Activity of human β-defensin 3 against metallo-β-lactamase-producing *Pseudomonas aeruginosa* strains

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Objectives: We sought to evaluate the bactericidal effect of human β-defensin 3 against metallo-β-lactamase (MBL)-producing *P. aeruginosa* clinical isolates.
Materials and Methods: A total of 15 unique multidrug-resistant *P. aeruginosa* strains were tested that originated from relevant specimens of patients hospitalised at our hospital. Bacterial identification and antimicrobial susceptibility testing was performed with the VITEK 2 automated system. According to their drug susceptibilities all clinical isolates were categorised into 3 distinct resistance phenotypes. Metallo-β-lactamase (MBL) production was detected with the use of Etest MBL strips, according to the manufacturers' instructions. To determine the MBL types all isolates were subjected to PCR analysis to confirm the presence of blaVIM and blaIMP genes. The bactericidal activity of recombinant hbD-3 was assessed by the liquid microdilution assay in the presence of 10mM sodium phosphate buffer, as previously described. Briefly, exponentially growing bacteria (inoculum density of 1×10^4 cfu/mL) were exposed to different concentrations of hbD3 (0.5 ug/mL and 0.25 ug/mL). Following incubation for 20 min at 37ºC, 0.1 ml of each sample was plated onto McConkey agar plates. Bactericidal effect was expressed as percentage of reduction in numbers of viable bacteria after 18 to 24 hr incubation at 37ºC in ambient air.
Results: At a concentration of 0.25 ug/mL, the peptide produced a reduction rate ranging from 55% to 95.4% whereas the use of 0.5 ug/mL of hbD-3 resulted in 68% to 100% killing. The significant variability in the bactericidal effect observed, appears to be independent of the isolates' resistance phenotypes.
Conclusion: Despite the relatively low concentrations used, hbD-3 exhibited bactericidal activity against MBL-producing *Pseudomonas aeruginosa* nosocomial strains and therefore it would be an attractive alternative to current theurapeutic agents.

O439 In vitro activity of temocillin and other antimicrobial agents against extended-spectrum-β-lactamase-producing Enterobacteriaceae isolated from patients hospitalised in Belgian intensive care units

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Objectives: Temocillin is a 6-a-methoxy-derivative of ticarcillin with increased stability to most β-lactamases including AmpC and extended-spectrum types (ESBL). Data concerning its activity against Enterobacteriaceae clinical isolates are however scarce. The aim of this study was to evaluate the in vitro activity of temocillin against 652 clinical isolates of Enterobactericaeae collected from patients hospitalised in ICUs at seven Belgian university hospitals in 2005.
Materials and Methods: Each centre collected prospectively up to 100 unduplicated Enterobacteriaceae isolates from patients hospitalised in ICUs for >3 days in order to focus the collection on nosocomial pathogens. E-test MICs were determined for temocillin and five comparators (ceftazidime, piperacillin-tazobactam, meropenem, ciprofloxacin, and amikacin). The presence of ESBL was assessed by double combination disks (cefotaxime and ceftazidime) and by ESBL E-tests. ESBLs were characterised by IEF and PCR for bla genes of SHV, TEM, and CTX-M families and by DNA sequencing.
Results: The prevalence of ESBL-producing Enterobacteriaceae (ESBLE) averaged 12% but ranged between 3% and 29% in the different hospitals. A large diversity of ESBL types was observed within different species with the predominance of TEM-24 among *Enterobacter aerogenes*, CTX-M-1 group in *Escherichia coli*, and CTX-M-9 group in *Enterobacter cloacae* and *Klebsiella oxytoca*. SHV-derived ESBLs (SHV-5 and SHV-12) occurred less commonly and mainly in *E. cloacae* and *Klebsiella* spp.
Temocillin was active against >90% of the isolates, with MIC50 and MIC90 of 4 μg/mL and 16 μg/mL, respectively. Meropenem exhibited the best activity overall (susceptibility 99%; MIC50 and MIC90 of 0.064 μg/mL and 0.19 μg/mL) whereas ceftazidime (21% non-susceptibility) and ciprofloxacin (20% non-susceptibility) scored the worst. A high frequency of resistance to ceftazidime (89%) and to ciprofloxacin (72%) was observed especially among *E. aerogenes*. On the other hand, temocillin retained good activity against ESBL-producing *E. coli* (92%) but was found less active against some ESBLE and AmpC-derepressed producers (*E. aerogenes*, *Serratia marcescens*).
Conclusions: These in vitro data illustrate the wide variation in prevalence of ESBLE isolates in Belgian ICUs and support the usefulness of temocillin as therapeutic option for infections caused by ESBLE and other cephalosporin-resistant strains which are causing an increasing number of infections in critically-ill patients.

Keynote lectures

K443 Systems biology in clinical microbiology and infectious diseases

J. Haas (Munich, DE)

Systems biology is a fast developing research area in life sciences which combines both experimental and theoretical disciplines and which investigates all components of complex biological systems (for example all proteins of a cell). It collects large biological datasets by novel, high-throughput-based technologies and develops computational tools for its bioinformatic analysis. It thus allows a holistic view on complex biological systems and to set up improved biological models, particularly if several different experimental parameters are integrated (for example protein interaction and expression profiling data). Although Systems biology is usually based on screening assays and thus starts without bias, in contrast to hypothesis-driven research, in most cases it eventually leads to a multitude of novel working hypotheses. Particularly interesting are hypotheses derived from bioinformatic concepts like network emergence, robustness and modularity, since it is currently completely unclear if and how they translate into biology.
Systems biology-based approaches in infectious diseases are even more complex, as they investigate the interactions between the components of two distinct biological systems, pathogen and host. On the host side, cellular components which directly deal with the control of the infection, i.e. the innate and adaptive immune system, are of particular interest. For the understanding of the pathogenesis, pathogen products counteracting these cellular components are similarly important. By integrating all components of the immune system, Systems biology-based approaches might be able to generate better disease models than previous reductionist approaches focusing on only one or few pathogenic aspects. For example, in herpesviruses, which cause persistent latent infections, both the innate as well as the adaptive immune system and their components play a role at different time points after infection and thus have to be considered to fully understand their pathogenesis.
Recently developed technologies in molecular biology allowing genome-wide analyses are one of the prerequisites for Systems biology-based approaches. For example, novel sequencing technologies which are powerful enough to evaluate whole genomes within short time are able to detect inter-individual polymorphisms. There is growing evidence that genetic variations on both the host and the pathogen side are often crucial for the outcome of infectious diseases. A variety of other experimental systems have been developed during the last decade and are meanwhile sufficiently potent to allow genome-wide analyses, including microarrays for transcriptional or protein expression profiling, genetic screening systems like the yeast-two-hybrid system used to identify pairwise protein interactions and novel mass spectrometry approaches used for proteomics and metabolomics.
In medicine, Systems biology is currently still largely considered as a basic research area with little practical relevance. However, it will lead to substantial changes in medicine within only a few years, not just because Systems biology-based disease models will considerabley improve our understanding of the pathogenesis and will accelerate and rationalise drug discovery, but also since the ability to determinate individual genetic traits and availability of multi-parameter diagnostics will eventually lead to a much more personalised medicine, in which therapeutical interventions are tailored to single individuals.

K444 Clinical implications of viral evolution and variability

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Human immunodeficiency virus represents the best model of evolution on earth. Due to the high rate of replicative cycles, and the error-prone polymerase (able to make about 1 error out of 10,000–30,000 nucleotides), the entire genome of HIV can be changed every day. Based on these factors, it has been calculated that HIV genome varies in one day more than the variability of influenza virus (another highly variable virus) in 10 years, and more than the variability of the human genome during the whole history of mankind.
Despite this immense potentiality, only few mutations that are randomly produced during the daily replicative cycles are fixed within the viral genome. This is because of a bottleneck phenomenon related to the constraint of target cells (unable to support the replication of all vira strains daily generated) and because the large majority of the mutations generate variants whose replicative capacity is very low/absent, or in any case lower than the one that characterised the wild type strain. For this reason, the number of quasispecies is limited if the environment (that is immune and pharmacological pressure) does not change.
Of course the situation changes unpo environment change. In case of therapeutic pressure, the ability of the virus to rapidly deselect the wild-type strain in favour of a resistant mutant is a function of the strength of drug regimen. In case of fully suppressive therapy, the virus is unable to generate and/or select new variants, the wild-type strain remains prevalent and the therapy is highly successful. By contrast, if therapeutic pressure is inconsistent, erratic, or not sufficiently potent, the virus continues its replicative cycles under drug presence, and this

represents the best environment to select mutant-resistant strains. The rapidity with which this selection occurs is a function of the genetic barrier of each drug, that in turn is related to the number of mutations occurring to generate a new variant fully resistant to each drug. A low genetic barrier means few (1 or 2) mutations are required to generate resistant variants, that will occur rapidly. A high genetic barrier (5–6 or more mutations occurring in sequence) makes more difficult and slower the process of selection of a resistant strain.
These data have a strong clinical implication, in view of the impossibility (at least today) to eradicate virus infection from the body. Thus, anti-HIV therapy has to be started before the damage of the immune system is too advanced, and must be strong and consistent. For this reason, until today, at least three drugs rationally combined are needed to achieve this result. A great effort has to be dedicated by physicians and nurses to the achievement of a very high level of adherence, that represents a key factor to maintain a long-lasting protection of HIV-disease progression and a good quality of life.

Atypical mycobacterial infections

O448 Non-tuberculous mycobacteria in a Spanish teaching hospital during a five-year period

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Objective: to know the prevalence of nontuberculous mycobacteria (NTM) isolated in a teaching hospital during a five-year period.
Methods: a total of 21,186 specimens obtained from patients attended at Area 2 of Madrid were studied. Samples were processed by standard methodology and were inoculated onto solid (Lowenstein and Coletsos) and liquid media using a semiautomatic equipment (MGIT®). *Mycobacterium tuberculosis* complex, *M. avium* complex, *M. gordonae* and *M. kansasii* strains were identified by DNA probes (Accuprobe®) and the rest were studied by biochemical tests, PCR-RFLP or sent to a Reference Laboratory.
Results: a total of 645 mycobacteria were isolated from 324 patients. *M. tuberculosis* complex was the mycobacteria most frequently isolated: 431/645 (66.8%). NTM isolates (336 strains from 111 patients) are summarised in the table.

Non-tuberculous mycobacteria isolated

Mycobacteria	Isolates	Patients
M. avium complex	122	5
M. fortuitum	16	14
M. gordonae	15	11
M. kansasii	14	5
M. abscessus	14	3
M. simiae	16	14
M. chelonae	7	6
Others*	13	12

Others: *M. lentiflavum* (3), *M. mucogenicum* (2), *M. septicum* (1), *M. thermoresistibile* (3), *M. xenopi* (3) and *M. porcinum* (1). A small percentage of NTM (3.1%) was isolated from non-respiratory samples. In 35 patients (31.5%), NTM were isolated in at least two specimens.

Conclusions:
1. NTM involve an important burden in the clinical mycrobiology laboratory.
2. *Mycobacterium avium* complex is the NTM most frequent in our study.
3. Although the isolation of NTM from repeated specimens of the same patient is not rare (31.5%), the clinical relevance of these data remains controversial.

O449 Incidence of disseminated *Mycobacterium avium* complex infection in the USA compared to three European regions

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Objective: The incidence of invasive *Mycobacterium avium* complex (MAC) infections in HIV-infected patients differs widely in studies from different geographical regions. Comparative studies have not been done yet. We analysed the incidence of atypical mycobacteriosis in different geographical regions using a large dataset of HIV-infected patients enrolled in a randomised trial of MAC prevention. Monthly blood cultures were taken in this trial, giving the opportunity to estimate incidence rates.
Methods: Data from a multinational, multicentre trial of prevention of MAC infection with clarithromycin in HIV-infected patients with less than 100 CD4 cells were analysed (Pierce, NEJM 1996). We estimated the incidence of disseminated MAC infection (defined by the first positive blood culture with MAC) in 4 different geographical regions (USA, UK, France and Germany) in a Cox-proportional hazard model, stratifying by use of prophylaxis on an intention-to-treat analysis.
Results: 682 patients (pts) were included in the study (USA 333, UK 36, F 175, G 136). Patient characteristics (age, gender, body mass index, CD4 cells/mcl) between these regions were well comparable. Overall 19% of pts from the USA, 25% from the UK, 10% from France and 8% from Germany developed invasive MAC-infection. In a multivariate Cox proportional hazard analysis corrected for CD4 cell counts at baseline the risk ratio (RR) of disseminated MAC for patients from France was 0.24 ($p < 0.01$) and for pts from Germany 0.25 (0.01) compared to the USA. The incidence in the UK was not different from that in the USA.
Conclusions: The incidence of MAC infections in HIV-infected patients differs widely between the USA and different European regions. This may have important consequences for preventive strategies.

O450 Infections due to non-pigmented rapidly growing mycobacteria in Madrid, 2005

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Objectives: To determine the incidence, clinical significance, and epidemiology of the isolates of non-pigmented rapidly growing mycobacteria (NPRGM) in Madrid, Spain, during 2005.
Methods: Patients with isolates of NPRGM were selected prospectively during the year 2005. All the isolates were identified according recommended biochemical tests and PCR-RFLP Analysis of the hps65 gene. Clinical charts were reviewed according to a predefined protocol, and clinical significance was evaluated according to accepted criteria. Molecular epidemiology was analysed using RAPD technique with 3 primers (OPA-2, IS 976FP and INS 2). Identity between strains was considered when they shared identical pattern between them with all the primer sets.
Results: NPRGM were identified in 70 patients (incidence: 1.51 cases/100,000 inhabitants). 74 strains were available for evaluation. The identified species were *Mycobacterium abscessus* (5 cases), *M. chelonae* (9 cases), *M. fortuitum* (40 cases), *M. peregrinum* (10 cases), *M. mageritense* (5 cases), and *M. mucogenicum* (1 case). The isolates were considered clinically significant in 18 cases (25.7%, incidence: 0.39 cases/100,000 inhabitants): 4 *M. abscessus*, 5 *M. chelonae*, and 9 *M. fortuitum*. 6 cases were respiratory infections (4 in patients with cystic fibrosis), 7 were nosocomial infections (3 catheter-related bacteraemia), 2 were soft-tissue abscesses, 1 canaliculitis, and 1 bacteraemia in an HIV patient. No cases were detected due to *M. peregrinum*, *M. mucogenicum*, or *M. mageritense*. The strains were isolated mainly from respiratory samples (56 cases), being significant only in 6 of these cases (10.7%), whereas clinical significance was considered in 12 cases out of 16 patients with nonrespiratory isolates

(75%) ($p < 0.001$). RAPD analysis showed no relationship from different cases.

Conclusions: NPRGM could be responsible of clinical syndromes in 1/4 of the cases, and this association was different for the species studied. Non-respiratory isolates were significant in 3/4 of the cases. The predominant species in our environment is *M. fortuitum* (57.1% of cases), although the most significant one was *M. abscessus*. No relationships among isolates from independent cases were detected, suggesting transmission routes other than interhuman.

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O451 *Mycobacterium szulgai* causes tuberculosis-like disease in Zambia

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Introduction: In Africa, the diagnosis of tuberculosis is almost invariably based on the microscopic examination of Ziehl–Neelsen stained clinical material. However, not only *Mycobacterium tuberculosis*, but also non-tuberculous mycobacteria (NTM) yield a positive result in microscopic examination for acid-fast bacilli (AFB). Furthermore, a significant part of the patients, especially HIV-positives, may represent AFB-negative, but culture-positive mycobacteriosis.

Objective: To investigate the clinical relevance of isolation of NTM in Africa, in the light of the increasing prevalence of HIV.

Methods: In Sesheke, Zambia, 64 (HIV positive and negative) patients, who were chronically ill for more than two weeks, were included in the study. Sputum was collected and cultured for mycobacteria using Mycobacteria Growth Indicator tubes. The isolated Mycobacterium cultures were identified by 16S rRNA gene sequencing.

Results: Thirty out of 64 (47%) patients yielded positive Mycobacterium cultures that were identified as *M. tuberculosis* (8 times), *M. szulgai* (7), *M. avium-intracellulare* (3), *M. simiae* (1) and *M. terrae* (1). Ten isolates were not suitable for identification due to contamination, re-culture problems, etc. Thirteen of the 30 culture-positive patients (43%) were also positive in microscopic examination, including four patients with NTM infections. Especially the patients infected by *M. szulgai* manifested symptoms highly similar to regular tuberculosis caused by *M. tuberculosis*. DNA fingerprinting analysis revealed four different patterns among the seven *M. szulgai* isolates, excluding the possibility of a laboratory cross-contamination or a common source of infection. Only two out of seven patients with a *M. szulgai* infection responded well to treatment by tuberculostatics.

Conclusion: The contribution of NTM, and especially of *M. szulgai*, to tuberculosis-like diseases in both HIV positive and negative patients in Africa may be underestimated.

O452 Administration of TNF-α did not inhibit *Mycobacterium avium* infection in the mouse lung

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Objective: *Mycobacterium avium* causes chronic and progressive respiratory infection. TNF-α plays a central role in innate immunity against mycobacteriosis. Although administration of TNF-antibody has been reported to cause mycobacteriosis including non-tuberculous mycobacteriosis, study concerning administration of TNF-α in non-tuberculous mycobacteriosis remains insufficient. In this study, we investigated the effect of TNF-α administration in *M. avium* infection in mice.

Methods: Clinically isolated strains of *M. avium* were used. Proliferation of *M. avium* within peritoneal macrophages in the existence of recombinant human TNF-α were examined. Wild type of C57Bl/6 mice, perforin-deficient mice or TNF-α overexpression mice were administered *M. avium* (1×10^7 cfu/body) intratracheally. Recombinant human TNF-α (10 ug/body) was injected into wild mice or perforin-deficient mice on day 0 and day 7 after *M. avium* administration. Mice were sacrificed on day 21, 60 after *M. avium* administration. The lung homogenates were inoculated on Middlebrook 7H10 agar plates for counting the number of colonies. Tissue sections of the lungs were stained by hematoxylin and eosin or Ziehl–Neelsen methods.

Results: *In vitro study*: administration of recombinant human TNF-α inhibited proliferation until day 3, but not day 7. *In vivo study*: administration of recombinant human TNF-α did not inhibit *M. avium* infection based on the lung histology and bacterial number in the lungs of perforin-deficient mice. In TNF-α transgenic mice, *M. avium* induced several lymphoid tissues proliferation. Bacterial proliferation in the lung was not inhibited compared to wild type mice.

Conclusion: Both exogenous and endogenous TNF-α administration did not attenuate *M. avium* infection in vivo. The present data indicate that the role of TNF-α against *M. avium* is different from that of *M. tuberculosis*.

New antimicrobial compounds overcoming common resistance mechanisms

O453 Mercapto-phosphonate compounds as broad-spectrum inhibitors of the metallo-β-lactamases

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Objectives: One of the emergent factors for the β-lactam antibiotic resistance of pathogenic bacteria is the production of metallo-β-lactamases (MBLs), which are able to hydrolyse the β-lactam ring in a broad spectrum of substrates, particularly the carbapenems. MBLs have been divided into three different sub-classes B1, B2 and B3 based on sequence similarities [1]. In this report, we investigated the inhibitory effect of mercapto-phosphonate derivatives against MBLs.

Methods: The laboratory of P Metzner (University of Caen, France) synthesized 12 different mercapto-phosphonate compounds with the ability to inhibit the subclass B1 VIM-4, the subclass B2 CphA and the subclass B3 L1 MBLs respectively. Consequently, we determined the competitive inhibition constant (Ki) as described by DeMester et al. [2]. We also measured the minimal inhibition concentration (MIC) for *Escherichia coli* recombinant strains producing VIM-4, CphA or L1, for ampicillin or imipenem in the presence or absence of mercapto-phosphonate compounds.

Results: In the present study, we show that all the mercapto-phosphonates, with the exception of compound 1a, behaved as good competitive inhibitors ($Ki < 15\,\mu M$) for CphA. Their activities against the sub-classes B1 and B3 enzymes were more contrasted. In addition, the presence of free Zn++ abolished the inhibitory activity of compound 2b. The compound behaving as zinc chelator could explain this phenomenon. Nevertheless, the (2-sulfanylphenyl) phosphonic acid, the (4-bromophenyl)(sulfanyl)methyl phosphonic acid and the [(2,4-dichlorophenyl)(sulfanyl)methyl] phosphonic acid were good inhibitors ($Ki < 15\,\mu M$) against the different studied enzymes and can be used as leads to the synthesis of new MBL inhibitors. Our tests indicated that the presence of compounds 2b, 4a and mgfg decreased the MIC value for imipenem.

Conclusion: In this study, we show that members of the phosphonates group are able to enhance the inhibition of Zn β-lactamases. This is the first report of new inhibitors possessing a strong activity against the different sub-classes of MBLs.

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O454 The activity of NXL101 against community-associated methicillin-resistant *Staphylococcus aureus*

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Objectives: To evaluate the novel topoisomerase inhibitor NXL101 against community-associated (CA) methicillin-resistant *S. aureus* (MRSA).
Methods: A total of 54 CA-MRSA were investigated: 48 being recent clinical isolates from community sources within Europe and further defined as CA-MRSA by cefoxitin 30 μg disc zone of inhibition ⩽19 mm, possessing staphylococcal cassette chromosome mec type IV or IVa and having full susceptibility to gentamicin (GEN). The remaining 6 were CA-MRSA reference isolates from the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA) collection. MIC was determined for NXL101, GEN, azithromycin (AZI), clindamycin (CLI), dalfopristin–quinupristin (D–Q), daptomycin (DAP), levofloxacin (LFX), linezolid (LZD) and vancomycin (VAN) by CLSI broth microdilution.
Results: Summary MIC data are shown in the table. NXL101 was the most active agent against CA-MRSA. A high percentage of resistance was observed to LEV and AZI, but full susceptibility was shown to most other agents apart from CLI.

	Percentage			MIC (mg/L)			
	Susceptible	Intermediate	Resistant	MIN	50%	90%	MAX
NXL101	–	–	–	⩽0.015	0.06	0.12	0.5
AZI	44.4	0	55.6	0.25	>64	>64	>64
CLI	75.9	0	24.1	0.06	0.12	>8	>8
D–Q	100	0	0	0.25	0.25	1	2
DAP	100	–	–	0.12	0.5	1	1
GEN	100	0	0	0.12	0.25	0.5	0.5
LEV	22.2	0	77.8	0.12	8	16	>32
LM	100	–	–	0.5	2	2	4
VAN	100	0	0	0.5	1	1	1

Conclusion: NXL101 showed excellent activity against CA-MRSA, and may offer a viable alternative for the treatment of CA-MRSA and other *S. aureus* infections in the future.

O455 Comparative dose studies of API-1252 and linezolid against hospital-acquired and community-acquired methicillin-resistant *Staphylococcus aureus* in a murine thigh model

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Objectives: API-1252 is a novel antimicrobial, with a mechanism of action targeting fatty acid biosynthesis, currently in development as a new class of oral and intravenous anti-staphylococcal agents. In this study, comparative single-dose response experiments were performed with API-1252 and linezolid to assess their quantitative oral efficacy against hospital-acquired (HA)-MRSA and community-acquired (CA)-MRSA.
Methods: MICs were determined by CLSI guidelines. Single dose PK studies were conducted. The thighs of neutropaenic mice were inoculated (~10^6) with a single isolate of HA-MRSA or CA-MRSA. API-1252 and linezolid were administered orally in single doses ranging from 1 to 300 mg/kg. CFU were determined in infected thighs 24 hours post-dose. Efficacy was determined as the change in CFU/thigh at 24 hours versus the 0 hour controls.
Results: MICs of API-1252 and linezolid against both isolates were 0.004 and 2 mg/L, respectively. For the HA-MRSA, CFU reduction was seen with API-1252 doses of ⩾10 mg/kg. While the 10 mg/kg dose displayed a variable effect for the CA-MRSA, doses of ⩾40 mg/kg of API-1252 resulted in sustained antibacterial activity. Linezolid doses of ⩾100 mg/kg were required to maintain sustained antibacterial activity over the 24 hour exposure period for both isolates. With both MRSA isolates, the 80% maximally effective dose (ED80, mg/kg), ED50, and ED5 were 4–20× lower for API-1252 when compared to linezolid. Pharmacodynamic index correlations showed that the fAUC/MIC of API-1252 was the best predictor of efficacy. For both MRSA strains the API-1252 fAUC/MIC ranges resulting in the ED80, ED50, and ED5 were 32–69, 29–32, and 6–25, respectively. These ED values were similar to that observed with MSSA for API-1252 (ICAAC 2006, Abstract F1-759).
Conclusion: These data demonstrate the superior dose-response of API-1252 as compared to linezolid against HA-MRSA and CA-MRSA in the mouse thigh model. fAUC/MIC appears to best characterise the PD profile of this novel agent. The low efficacious fAUC/MIC values support the further development of API-1252 as a novel oral and intravenous agent for challenging staphylococcal infections.

O456 Deletion analysis of LysK, a bacteriophage-derived protein with anti-MRSA activity

M. Horgan, G. O'Flynn, G.F. Fitzgerald, A. Coffey, O. McAuliffe, R.P. Ross (Fermoy, Cork, IE)

The emergence of multi-drug-resistant pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA), combined with the absence of new antibiotics from the pharmaceutical sector demands that alternative anti-MRSA agents are scientifically evaluated and developed as a matter of urgency. Recent studies have shown the enormous potential of the use of phage endolysins as potential therapeutics.
Objectives: To characterise the staphylococcal phage K-derived protein, LysK, a bifunctional endolysin with antimicrobial activity against MRSA, with a view to identifying the domain or domains responsible for lytic activity.
Methods: The lysK gene was PCR-amplified from phage K cDNA and cloned into the pTOPO (Invitrogen) vector. The domain architecture of LysK was studied by performing deletion analysis on the intact LysK protein using PCR and/or slicing by overlap extension (SOEing) PCR on this pTOPO clone. To assess the activity of LysK deletion derivatives, constructs were recreated in the pQE60 (Qiagen) expression system. SDS-PAGE and zymogram assays were used to visualise the activity of LysK and its deletion derivatives in pQE60 and to assess which domain(s) the lytic activity of LysK could be ascribed to.
Results: Bioinformatic analysis of LysK (495 amino acids) suggests that it has a modular structure, containing two peptidoglycan hydrolase domains, CHAP (endopeptidase activity) and Amidase_2 (N-acetyl-muramoyl-L-alanine amidase activity), at the N-terminus and a cell-wall binding domain at the C-terminus (SH3b). Analysis of deletion derivatives of LysK confirmed that while Amidase_2 and SH3b domains had no significant activity alone, the CHAP domain was as active as the intact LysK, displaying an identical lytic spectra when examined by zymographic assays. While attempting to define the smallest possible functional CHAP domain with antimicrobial activity, we found that the endopeptidase activity associated with the CHAP domain is contained within the first 161 amino acids of LysK. Further deletions resulted in a complete loss of antimicrobial activity. The 161 amino acid truncated CHAP has been found to be active against the main MRSA strains emerging in hospitals in the local area.
Conclusion: The CHAP domain of LysK has catalytic properties similar to that of LysK itself, highlighting the possibility of creating chimeric proteins with different substrate specificities, thus expanding their lytic capabilities.

O457 The activity of NXL101 against fluoroquinolone-resistant clinical isolates of *Staphylococcus aureus*

I. Morrissey, J. Lowther, M. Soltani, J. Northwood (London, UK; Romainville, FR)

Objectives: To evaluate the novel topoisomerase inhibitor NXL101 against fluoroquinolone-resistant (FQR) *S. aureus* (SA).
Methods: MIC against 39 FQR SA and the control strain ATCC29213 (Cont) was determined for NXL101, levofloxacin (LFX), ciprofloxacin (CFX), moxifloxacin (MFX) and gemifloxacin (GFX) by CLSI broth microdilution. Topoisomerase gene (grlA, grlB, gyrA & gyrB) quinolone resistance determining regions (QRDRs) were amplified and sequenced and the effect of reserpine (RES, 20 mg/L) on MIC (i.e. the role of FQ efflux) was evaluated to determine mechanism(s) of resistance.
Results: MIC, QRDR and efflux data are shown in the table. Most FQR SA possessed combined GrlA (S80F)–GyrA (S84L) changes. Others had additional changes in GrlA, GyrA or GrlB, but none were found in GyrB. The effect of RES on CFX MIC was variable, but this did not affect FQ MIC as much as QRDR changes. Other FQ were less affected by RES than CFX. NXL101 MIC ranged from 0.03 to 1 mg/L (mode 0.06/0.12 mg/L) and was not affected by any QRDR changes. Some RES effect was observed with NXL101, but most FQR SA had similar NXL101 MIC to the Cont strain (for which NXL101 MIC was not affected by RES).

No. of	AA Change			Efflux	MIC range (mg/L)				
Isolates	Grl A	GrlB	GyrA		NXL	MFX	GFX	LFX	CFX
10	S80F	–	S84L	++	0.06–0.25	2	2–8	8–16	32–512
5	S80F	S84L	+++	0.06–1	2	2–8	8–16	256	
5	S80F	S84L	N	0.12–0.25	2–4	2–8	8–16	16–256	
3	S80F	S84L	+	0.06–0.5	2–4	2–8	8–16	32–256	
2	S80F	D432V	S84L	++	0.25	8	16–32	32	256
1	S80F, P144S	E422D	S84L	++	0.12	4	8	8	256
1	S80F, P144S	E422D	S84L	+	0.12	4	8	16	256
1	S80F, P144S	–	S84L	N	0.06	2	4	16	16
1	S80F, E84K	–	S84L	+++	0.06	8	8	16	256
1	S80F, E84K	–	S84L	++	0.12	8	8	32	256
1	S80F, E84K	–	S84L	+	0.06	4	8	16	128
1	S80F, E84K	–	S84L	N	0.06	8	16	32	256
1	S80F, E84K	–	S84V	N	0.06	8	4	16	256
1	S80F, E84G	–	S84L	++	0.06	8	32	32	128
1	S80F	D132N	S84V	++	0.03	4	2	16	128
1	S80F	–	S84L, G106D	+	0.5	2	2	8	16
1	S80Y	D432H	S84L	+++	0.06	4	2	16	256
1	S80Y	D432N	S84L	++	0.12	4	8	32	256
1	S80Y	–	S84L	++	0.25	2	2	8	16
Cont	–	–	–	N	0.06	0.06	0.015	0.25	0.25

[a] Efflux: effect of RES on CFX MIC: +++ (4–6 dilutions decreased), ++ (2–3 dilutions decreased), + (1 dilution decreased), N (no effect).

Conclusion: NXL101 retained activity against highly FQR SA exhibiting a range of QRDR mutations and CFX efflux. NXL101 must target topoisomerases in a manner distinct to that of FQs.

Harnessing the host response for anti-infective therapy

S459 Controlling pathogenic bacteria with phage lytic enzymes

V.A. Fischetti (New York, US)

Bacteriophage lytic enzymes are highly evolved molecules used by the phage to quickly destroy the bacterial cell wall to release phage progeny. We have exploited the rapid and lethal action of these enzymes to destroy pathogenic and biological warfare bacteria on mucous membranes and in blood. These enzymes in general are specific for the species or strain from which they were produced, thus avoiding destruction of the surrounding normal commensal organisms found on mucosal surfaces. We now have enzymes that are specific for *Streptococcus pyogenes*, *S. pneumoniae*, *Bacillus anthracis*, *Staphylococcus aureus*, *Enterobacter faecalis/E. faecium* and group B streptococci. Our results show that in vitro 10^7 bacteria can be reduced to sterility seconds after enzyme contact. In animal model experiments, we were able to colonise mice with either streptococcal or pneumococcal species (orally or nasally) and remove these completely with phage enzymes delivered to these sites using a single enzyme dose. In a septicaemia model with *S. pneumoniae*, bacteria are reduced by >2-logs from the blood of infected animals with a single intravenous dose of enzyme. A lytic enzyme called PlyG from the gamma-phage of *B. anthracis* was specific for all worldwide isolates of *B. anthracis*. The enzyme specifically killed *B. anthracis* with no effect on other bacilli or other organisms. When >1 LD100 of *B. anthracis* bacilli were delivered i.v. to mice we observed a progression of symptoms, leading to survival of only 10% of animals followed for 12 days. When PlyG was injected i.v. 15 min after infection, a significant therapeutic effect was observed in which 90% of the mice recovered fully. Resistance to the enzymes has not been found nor do antibodies neutralise their activity. Furthermore, a combination of antibiotic and enzyme has been shown to work synergistically resulting in efficient lethal activity in cases of antibiotic resistant bacteria. Thus, phage lytic enzymes are a new reagent that may be used in hospitals, nursing homes and the general population to control antibiotic resistant pathogenic bacteria in blood and on mucosal surfaces, offering a capability previously unavailable.

S461 Two-component signal transduction systems of pathogenic bacteria as targets for anti-infective therapy

K. Stephenson (Leeds, UK)

Two-component signal transduction systems are ubiquitous in bacteria and are woven within the fabric of the regulatory processes that are used to sense environmental change and respond accordingly. These systems allow the bacterial cell to continually re-programme patterns of gene expression rapidly and in defined ways to bring about cellular adaptation as a direct consequence of specific environmental signals. To achieve this function two-component systems are composed of a signal ligand-responsive sensor histidine kinase that is usually an integral membrane protein, and a response regulator that is often a transcriptional regulator. Signal ligands are sensed by the sensor histidine kinase resulting in ATP-dependent autophosphorylation and the subsequent transfer of the phosphoryl moiety to the cognate response regulator influences its DNA binding and transcriptional activity, and therefore gene expression.
For bacterial pathogens these signal transduction pathways are frequently key elements in the regulation of a myriad of virulence responses that facilitate colonisation, survival and persistence within the host environment and at specific host sites. Examples of the types of virulence attributes modulated by two-component systems include biofilm formation, quorum sensing, resistance to host defence peptides, and secreted toxin production. In addition, two-component systems that are essential for bacterial viability are known to exist. For these and other reasons two-component signalling systems and their variants have been recognized as targets for the development of anti-infective drugs.
In this presentation the features of two-component systems that make them potentially attractive targets for the development of novel anti-infectives will be discussed. The mechanisms of action and limitations of some classes of previously developed two-component system inhibitors will also be discussed, as will the current and future perspectives for the development of selective inhibitors of these bacterial signalling systems.

Endemic MRSA – is control possible?

S464 If the question concerns MRSA, the solution contains detergent

S. Dancer (Glasgow, UK)

The global increase of methicillin-resistant *Staphylococcus aureus* (MRSA) has generated much attention over the last decade. The public have linked the so-called ‘superbug’ with their experience of dirty hospitals, but the precise role of cleaning in the control of this organism

is unknown [1]. There is some support for a link between poor hygiene in hospitals and MRSA, since its epidemiological characteristics permit survival in the clinical environment as well as make it potentially vulnerable to the cleaning process [2]. Unfortunately, we cannot assess the risk of acquiring MRSA from the clinical environment because there is no way of measuring the effect of cleaning. Perhaps it is time to introduce microbiological standards for surface levels in hospitals [3]. This would not only allay concerns over the grading of hygiene by visual assessment, but would provide a means whereby the removal of dirt becomes an evidence-based science [3,4]. Without such evidence, the importance of a clean hospital will continue to remain speculative.
Even if the benefits of cleaning are well established, buffing the floors to a shine will not reduce the number of patients acquiring MRSA. Floors may form a repository for a variety of organisms but they do not play a major role in HAI [5] Pathogens are delivered to patients on hands, and it is more likely that contaminated hand-touch sites are a greater risk for MRSA acquisition [3,6]. Such areas should be prioritised when managing cleaning schedules, since a targeted approach to hospital cleaning might be a useful control factor for MRSA [7].
We will never be able to guarantee consistent hand hygiene nor a sustained reduction in antibiotic consumption. Improvements in cleaning, however, are not insurmountable [1]. More research on the association between MRSA and environmental hygiene is urgently required. Basic cleaning could deliver significant cost benefits in MRSA control and could ultimately be our only defence against this organism.

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S465 Decolonisation strategies

J. Kluytmans (Breda, NL)

Carriage of MRSA is most often transient but can be persistent. In patients carriage is associated with an increased risk for the development of infection. Healthcare workers who carry MRSA may transmit the micro-organism to patients. To control transmission and prevent the development of infection, eradication of carriage may be indicated.
The decision to start decolonisation therapy should be carefully balanced with the risk for the development of resistance. Factors that are associated with increased failure rates are: the presence of wounds or other skin lesions, the presence of indwelling devices, multisite carriage and the presence of reservoirs at home. In individuals without these risk factors treatment with mupirocin nasal ointment is succesfull in the majority of subjects. It is often combined with the application of antiseptic showering. In individuals who fail on this topical treatment or with the presence of risk factors for failure, systemic antimicrobial treatment is indicated. Well-designed trials are not available so at present an evidence-based recommendation can not be made. The existing data indicate that a combination of two agents including rifampicin is preferred. The choice of the agents should be based on the susceptibility testing results. Treatment failure may be based on recolonisation from a persisting reservoir at home. Therefore, in patients who (repeatedly) fail on decolonisation therapy these sources should be sought for.

Foodborne infections: from starter to finish

S466 Food safety and monitoring of foodborne infections

F. Boelaert, S. Bronzwaer, S. Potier Rodeia, P. Makela (Parma, IT)

The European Food Safety Authority (EFSA) was set up in 2002 following a decade of food scares and a loss of confidence by the European public which led to a complete overhaul of the European Union food safety system and policies. EFSA's mission is to provide Risk Assessment on matters related to Food and Feed Safety and to communicate on these risks.
In 2005, twenty-four Member States, Iceland, Norway, and Switzerland submitted information on the occurrence of zoonoses, zoonotic agents, antimicrobial resistance and foodborne outbreaks to the European Commission and EFSA.
Campylobacteriosis was found to be the most frequently reported zoonotic disease in humans within EU. Reported *Campylobacter* cases increased by 7.8% compared to the previous year rising to an incidence rate of 51.6 cases per 100,000 people and to a total of 197,363 recorded cases. Salmonellosis remained the second most frequent zoonosis with 171,775 reported human cases, despite the fall by 9.5% to an incidence rate of 38.2 compared to 2004.
Salmonella was most often reported from fresh poultry and pig meat where proportions of positive samples up to 18% were detected. In table eggs, findings of positive samples ranged from 0% to 6%, but over the past 5 years, an overall decreasing trend in occurrence of *Salmonella* in the eggs was observed. In animal populations, *Salmonella* was most frequently detected from poultry flocks.
Salmonella, *Campylobacter* and viruses were the most important causes of reported foodborne outbreaks in 2005. Egg and bakery products were the most common reported sources of *Salmonella* outbreaks, whereas broiler meat was an important source for both *Salmonella* and *Campylobacter* outbreaks. Foodborne virus outbreaks were most often caused by drinking water, fruits and vegetables.
Relatively high proportions of *Campylobacter* and *Salmonella* isolates from animals and food were resistant to antimicrobials commonly used in treatment of human diseases. This is especially the case for resistance to fluoroquinolones in *Campylobacter* isolates from poultry. Foodborne infections caused by these resistant bacteria pose a particular risk to humans due to possible treatment failure.
When the results of the routine monitoring of laying-hen flocks are compared to the results from an EU-wide, fully harmonised *Salmonella* baseline study in laying-hen holdings, the prevalences in the baseline study are remarkably higher than those in routine monitoring. This reflects the different sensitivities of sampling scheme and sample types used and demonstrates that a harmonised protocol should be used when comparing the situation in one Member State with another.

S467 Food-borne *Yersinia* infections – from molecular pathogenesis to laboratory diagnosis

J. Heesemann (Munich, DE)

Yersinia enterocolitica and *Y. pseudotuberculosis* infections (yersiniosis) occur predominantly in the northern hemisphere. The clinical manifestations range from acute gastroenteritis to divers postinfectious sequelae, most notably reactive arthritis. The pathogenicity of these Gram-negative bacteria has been studied intensively during the last decades, resulting in identification and molecular characterisation of a set of chromosomally and extrachromosomally (plasmid pYV) encoded pathogenicity factors (PF). Here, the function of the PFs in the context of cell culture and animal infection models will be discussed.
The most intriguing PFs are the yersinia outer proteins (Yops) which are "microinjected" into host cells via a type 3 secretion system (T3SS) and function as "tranquilliser" towards the innate host defence. Several PFs of yersiniae have become prototypic members of growing families of PFs: such as the invasin, the trimeric autotransporter (TATA) *Yersinia* adhesin (YadA) and the siderophore yersiniabactin encoded by high pathogenicity island (HPI). The *Yersinia* invasin (Inv) is closely related to the intimins (EaeA) of enteropathogenic (EPEC) and enterohaemorrhagic *Escherichia coli* (EHEC). The *Yersinia* adhesin (YadA) is a trimeric autotransporter and member of the oligomer coiled-coil adhesin (Oca) family which comprises adhesins of *Haemophilus influenzae* (Hia), *Moraxella catarrhalis* (UspA) and other species. The yersiniabactin biosynthetic gene cluster of the HPI is widely spread among members of the family of Enterobacteriaceae and has become

a marker for extraintestinal pathogenicity, in particular in *E. coli*. The molecular analysis of the pathogenicity of *Yersinia* has not only improved our understanding of bacterial mechanism of invasion and persistence but has also provided us with new tools for laboratory diagnosis and prevention. Several PFs turned out to be useful antigens for (i) differentiation between virulent and avirulent *Y. enterocolitica*, (ii) detection of a class-specific serum antibody response (serological diagnosis) and (iii) vaccination. Moreover, the T3SS of yersiniae is suitable for delivery of antigens to antigen presenting cells and, thus, opens a new strategy for the design of oral live vaccine carrier strains.

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S468 Diarrhoea-causing *Escherichia coli* pathotypes – which genes to target for identification?

T. Pál, Á. Sonnvend (Pécs, HU)

In the past decades various groups of *Escherichia coli* associated with diarrhoeal diseases in man have been recognized. Currently, the most frequently identified pathotypes are the enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), enterohaemorrhagic (EHEC), enteroaggregative (EAEC) and diffusely adherent (DAEC) *E. coli* strains. Individual classes are defined on the basis of more or less characterised interactions between the bacterial cell and the host when inducing diarrhoea. There are several problems associated with the recognition of these strains in the laboratory. Easily detectable phenotypic markers sufficiently specific and sensitive to distinguish these strains from their non-pathogenic counterparts are not known. Although certain virulence factors (toxins, adhesins, invasins) are always, or often present in particular groups, the detection of their expression frequently goes beyond the capabilities of diagnostic laboratories. The use of molecular methods specific to genes of these factors, often in multiplexed PCR systems, is currently the most straightforward diagnostic approach. The detection of some of these genes (e.g. Shiga toxin, stx, or intimin, eae) can serve screening purposes but their identification may not prove the actual virulence of the isolate. Virulence factors can be shared by members of different pathotypes, and can be present in isolates recovered from healthy individuals or in non-pathogenic strains. For some classes it is still not clear which particular combination(s) of these genes are necessary to cause disease (e.g. EAEC), while in others the existence of subgroups with various pathogenic potential are already known to exist (e.g. EPEC, or Shiga toxin producing strains). Currently, the lack of straightforward diagnostic algorithms limits our knowledge on the epidemiology of diarrhoea-causing *E. coli*. Furthermore, more data on the incidence of these pathotypes should help to understand the role of the individual host's susceptibility in the outcome when encountering these strains.

S469 Quinolone resistance in the food chain

J. Vila (Barcelona, ES)

Since their discovery antimicrobials have been extensively used in livestock and poultry, with significant beneficial effects on food animal health and production efficiency. Most classes of antimicrobials used in animals, including fluoroquinolones, have human analogues. Fluoroquinolones (FQ) are used in animal production primarily for: (1) therapy; (2) prophylaxis; (3) infection control (metaphylaxis); and (4) growth promotion in healthy animals. In contrast to human medicine in which treatment is customarily directed at the patient, entire groups of animals may be treated with the use of medicated feed and/or water. Moreover, growth-promoting dosages are usually at low concentrations for extended time periods, therefore both practices are a potentially significant driving force in accelerating the emergence of resistant bacteria in these animals that can be transferred through contact or food to infect humans. The acquisition of quinolone resistance in Gram-negative bacteria is mainly due to chromosomal mutations either in topoisomerase genes (mainly gyrA and parC) or in genes associated with a decreased uptake or by increased efflux of quinolones. In addition, a plasmid conferring low levels of quinolone resistance linked to the presence of the qnr gene, which encodes a protein protecting DNA gyrase and topoisomerase IV from quinolones, has been reported. The above mentioned mechanisms have been described in both *Escherichia coli* and *Salmonella* spp. and generate an increased level of FQ resistance in a step-wise model, with the first mutation found either in the gyrA gene or in genes involved in efflux pump(s), such these producing an over-expression of acrAB. However, *Campylobacter* spp. easily acquire high levels of FQ resistance associated with a mutation in the gyrA gene, likely because this microorganism does not have topoisomerase IV. Several studies have demonstrated an association between FQ use in animals and the subsequent isolation of FQ-resistant bacteria from the same animal. Antimicrobial-resistant enteric pathogens can reach humans through direct animal contact, or more commonly, through ingestion of contaminated water or foods.

Three scenarios may be proposed by which the use of FQ in food animals could affect the treatment of diseases in humans: (1) FQ-resistant bacterial pathogens are selected, and food is contaminated during slaughter and/or preparation. After consumption of the food, these pathogens cause an infection that requires antibiotic treatment and therapy is compromised; (2) FQ-resistant bacteria non-pathogenic to humans are selected in the animal. When the contaminated food is ingested, the bacteria transfer FQ-resistance determinants, such as plasmid carrying the qnr gene, to other bacteria in the human gut, commensal and potential pathogens; and (3) FQs remain as residue in food products, which allow the selection of antibiotic-resistant bacteria after the food is consumed.

In conclusion, ongoing surveillance of the antimicrobial susceptibility profiles of foodborne pathogens is needed to identify emerging antimicrobial-resistant phenotypes within the food production continuum. Moreover, barriers to stop the dissemination of FQ or other antimicrobial-resistant bacteria from animals to humans should be improved.

Perspectives in tuberculosis

O470 Tuberculosis in the very old: a ten-year experience

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Objectives: It has been suggested that tuberculosis (TB) in the elderly is often atypical and difficult to diagnose. There is a lack of information about TB in people over 80 years of age. The aim of this study was to examine current clinical manifestations, time to diagnosis and outcomes in old ($\geqslant$65 years) and in very old ($\geqslant$80 years) patients with TB.

Methods: An observational study of prospectively collected data of consecutive patients with tuberculosis from January 1995 to December 2004 in a single institution. Patients under the age of 18 with immunodeficiency (HIV, transplant, infliximab use) or suspected imported disease (less than five years of residence in Spain) were excluded.

Results: Out of a total of 449 patients with documented TB, 319 adult patients were included; of these, 210 (65.8%) were under the age of 65 and 109 (34.2%) were $\geqslant$65 [27 (8.4%) were $\geqslant$80 years]. The mean age of the younger group was 37.1 (range 18–64), and that of the older group was 75.5 (range 65–98). Comorbidities (measured by Charlson index) were significantly associated with older age (1.43 vs. 0.43; $p < 0.001$). Pulmonary tuberculosis was more frequent in the younger group (73.8% vs. 49.5%; $p < 0.001$) and had a higher rate of cavitation in the chest X-ray (27.6% vs. 2.75%; $p < 0.001$). Extra-pulmonary and disseminated tuberculosis were more common in the elderly (50.4% vs. 26.1%; $p < 0.001$). No significant differences were observed between groups in time to diagnosis (65.34 vs. 59.44 days; $p = 0.66$). Drug toxicity was significantly higher in the elderly (22% vs. 9.8%; $p = 0.006$), mainly

due to hepatic toxicity. Tuberculosis-related mortality (30-day mortality) was higher in the elderly (18.3% vs. 1.6%; $p < 0.001$), mainly due to acute respiratory failure (30%). The subset of patients older than 80 years had a significant higher TB-related mortality (44.4% vs. 9.8%; $p = 0.01$) compared with those between 65 and 79 years. No significant differences in co morbidities or in clinical manifestations were observed.
Conclusion: Tuberculosis in the elderly and in the very old had a higher frequency of atypical features and disseminated TB, more adverse drug reactions and increased TB-related mortality. Data suggest that TB in the ninth decade has a mortality rate 2-fold higher than in the age group of 65 to 79 years.

O471 A risk of tuberculosis persists in patients treated with anti TNF-α antagonist therapy despite prophylactic guidelines: identification of main risk factors

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Introduction: Official guidelines for TB screening and chemoprophylaxis have been elicited in France (2002 modified in 2005) as in other countries for patients who begin TNF-α antagonist therapy. Our objective was to assess the impact of these guidelines on TB incidence in such patients.
Methods: A prospective cohort study involving 486 clinical departments in mainland France was designed by a multidisciplinary group (RATIO) to describe opportunistic infections, severe bacterial infections or lymphomas occurring in patients treated with anti TNF. In accordance with national health authorities, all cases are notified to RATIO.
All TB cases were validated by an experts committee. A case–control study (25 first TB cases matched with respect to gender and subjacent illness with 2 control patients for each case) was performed.
Results: We report 37 cases of tuberculosis that occurred within 2.5 years in patients treated with TNF antagonists in France (infliximab 18 cases, adalimumab 17 cases and etanercept 2 cases). Median age was 61 years (range 20–83). Median anti TNF treatment duration at onset was 33 weeks (2–231). Indication was rheumatoid arthritis in 25 cases, ankylosing spondylitis in 10 cases, Takayashu disease in 1 case. All patients were taking concomitant immunosuppressive agents: prednisone (15), methotrexate (13), salazopyrine (5) or azathioprine (4). No one had received antituberculous chemoprophylaxis before starting anti TNF therapy. Before anti TNF therapy, results of the intradermal tuberculin test (Mantoux test) was <5 mm in 18 patients, between 5 and 10 mm in 7 patients, >10 mm in 1 patient and not done in 3 patients. In the multivariate analysis, the case–control study identified the following risk factors of TB: age (OR = 1.05 [1.03; 1.07], $p = 0.02$), last anti TNF received (reference etanercept OR = 1; adalimumab OR = 14.6 [1.65; 129.0], $p = 0.01$; infliximab OR = 5.9 [0.74; 47], $p = 0.09$) and methotrexate treatment (OR = 0.28 [0.01; 1.08], $p = 0.01$).
Conclusion: Although national guidelines for TB prophylaxis have been elicited, TB remains a risk associated with anti TNF-α therapy. The risk of TB is higher for older patients and for those treated with adalimumab and infliximab than with etanercept.

O472 Assessment and management of tuberculosis infection in patients due to start anti-TNF-α treatment

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Objectives: To evaluate a protocol for assessment of tuberculosis (TB) infection in patients due to start treatment with anti-TNF agents and to evaluate the efficacy and safety of chemoprophylaxis in anti-TNF treated patients.
Methods: Prospective evaluation of all patients referred to the Tuberculosis Unit of a third-level teaching hospital (Jan 2003–Oct 2006), for assessment of TB infection prior to initiation of anti-TNF treatment. Diagnosis of TB infection was based on anamnesis for TB risk, two step tuberculin skin testing (TST) and chest X-ray. Patients were followed up throughout the period of chemoprophylaxis and adherence was monitored by determining isoniazid metabolites in urine (or urine colour when appropriate). A positive TST was defined as an induration ⩾5 mm.
Results: 210 patients, 64% women, mean age 52 years, were evaluated. Baseline illness: 60.5% rheumatoid arthritis, 13.8% cutaneous psoriasis, 13.3% psoriatic arthritis, 11% ankylosing spondylitis, and 1.4% Crohn's disease. Thirty-eight (80.2%) patients were on immunosuppressive treatment. Thirty-one (14.9%) patients had BCG-vaccination, 6 had been treated for TB, and 2 had prior positive TST. Of 201 patients who underwent TST, 84 (41.8%) resulted positive (59 and 25 in the first and second test respectively), and 117 (58.2%) resulted negative. Chemoprophylaxis was given to 79 (39.3%) patients: 78, isoniazid for 9 months and 1 rifampin for 4 months. Three patients (3.8%) experienced a 5-fold increase of transaminase level above the ULN. After a 237 patient–years follow up, one of the 130 patients who finally received anti-TNF treatment developed TB (0.42%; 95% CI: 0.01–1.71). His two-step TST resulted negative and he began on adalimumab. Five months later, TB developed.
Conclusion: Systematic and protocolised assessment for TB infection and its treatment when indicated is a reliable and useful method to prevent anti-TNF-associated TB. Our data show that two-step TST is helpful to detect TB infection in a significant number of patients. Prolonged treatment with isoniazid seems to be safe in anti-TNF treated patients.

O473 QuantiFERON-TB Gold In-Tube is more reliable in smear-negative tuberculosis

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Objectives: Interferon-gamma release assays (IGRA), such as the QuantiFERON®-TB Gold In-Tube (QFT-GIT; Cellestis Ltd., Carnegie, Australia), are increasingly used for the diagnosis of tuberculosis (TB). These tests exploit immunological mechanisms that normally contain *Mycobacterium tuberculosis* (Mtb) infecting the human host. Failed containment results in clinical TB and is associated with proliferating Mtb. We thus wondered if in this situation indeterminate or negative QFT-GIT results were associated with positive microscopy, thus serving as a surrogate for the bacterial burden.
Methods: Between November 2004 and October 2006, 67 consecutive patients with culture-confirmed TB were prospectively enrolled. Patients had received no or less than 14 days of antituberculous therapy (n = 58) or were recruited within two months since begining treatment (n = 9). Severe immunosuppressive conditions were present in 14 (21%). QFT-GIT was performed according to the manufacturers' instructions. Laboratory investigations included fluorescence microscopy for acid-fast bacilli, and culture on both liquid and solid media. Univariate and multivariate analysis were done in StatView® version 5.0 (SAS Institute Inc., Cary, NC).
Results: Overall, 52%, 36%, and 12% of the patients had pulmonary, extrapulmonary, or combined pulmonary and extrapulmonary TB, respectively. In one patient, no microscopy result was available as Mtb was only detected in blood culture. Microscopy was positive in 40/66 patients (61%; 95% CI: 49–72%); respiratory and extrapulmonary specimens were smear-positive in 30/39 (77%) and 10/27 (35%) of TB patients, respectively. Overall, QFT-GIT was positive in 51 (76%; 95% CI: 65–85%) TB patients; indeterminate or negative QFT-GIT results occurred in 6 (9%) and 10 (15%) TB patients, respectively. Age and gender did not influence the QFT-GIT test result. However, the likelihood of having a negative or indeterminate QFT-GIT test result was significantly and independently associated with a positive microscopy (odds ratio [OR] 5.8, 95% CI: 1.2–28.2%; $p = 0.03$) and with immune-suppression (OR 3.2, 95% CI: 0.91–11.4, $p = 0.08$).
Conclusions: To our knowledge this is the first study that demonstrates a significant association of smear-positive TB and negative or indeterminate QFT-GIT results. This association was independent of, and stronger than, the effect of concomitant immune-suppression. The finding has implications for the use of QFT-GIT in clinical practice.

O474 Utility of the IFN-γ assays using *Mycobacterium tuberculosis*-specific antigens for the diagnosis of latent infection in contacts of people with sputum smear-positive pulmonary tuberculosis

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Objective: To determinate IFN-γ response by specific T cells with QuantiFERON-TB GOLD (QFN-TB GOLD) (Cellestis, Australia) and T-SPOT.TB (Oxford Immunotec, United Kingdom) in contacts of people with sputum smear-positive pulmonary tuberculosis.
Materials and Methods: We included 91 individuals enrolled in contact tracing studies after exposure to a sputum smear-positive pulmonary tuberculosis case. Blood samples and isolated peripheral blood isolated mononuclear cells were stimulated with *M. tuberculosis*-specific antigens ESAT-6 and CFP-10. We determined the IFN-γ production in whole-blood supernatants samples by EIA with the QFN-TB GOLD assay and in mononuclear cells by ELISPOT with the T-SPOT.TB assay. Both IFN-γ tests were performed according to the manufacturer's instructions. Tuberculin skin test (TST) was administered by the Mantoux method using two tuberculin units of PPD RT23 (Statens Serum Institut, Denmark). Induration was measured after 48–72 h. Indurations higher than 5 mm were considered positive.
Results: We included 91 individuals with a exposure higher to 6 h/day, 62 of them with BCG vaccination scar. The percentage of positive results for TST, T-SPOT.TB and QFN-TB GOLD, in non-vaccinated and vaccinated individuals were the following: 71.4% and 94.9%; 70.4% and 47.3%; and 57.7% and 40.3%, respectively. The overall concordance between T-SPOT.TB and QFN-TB GOLD was higher (k = 0.632). Between vaccinated individuals that initiate a prophylaxis treatment, T-SPOT.TB was negative in 45.7% of cases, and QFN-TB GOLD in 59.2%.
Conclusions: (1) Both in vitro assays seem to have lower interference results with the BCG vaccination than TST, which suggest a higher specificity for QFN-TB GOLD and T-SPOT.TB assays. (2) Utilisation of these tests can help to reduce the number of unnecessary prophylaxes.

O475 Duration of exposure to a case of smear positive tuberculosis and rates of positive whole-blood interferon gamma test and tuberculin skin testing

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Objective: Duration of exposure to tuberculosis (TB) is a major risk factor for transmission of the disease. Current WHO guidelines, based on transmission of TB on aeroplane flights, recommend an 8 hour period as a cut off for screening. However this was before the routine use of IFN-γ assays. We compared Mantoux skin testing with QUANTIFERON-TB GOLD (QFN) in a hospital associated outbreak, stratifying patients by duration of contact.
Methods: Hospital contacts of the index case were screened in a sequential manner (close contacts sharing the same room for ⩾8 hours, contacts on the same ward for ⩾8 hours and contacts on ward <8 hours). Contacts were screened by TB specialist nurses with both Mantoux skin testing and QFN. A 15 mm cut off for reading Mantoux tests was used. Data on symptoms, duration of exposure and prior BCG vaccination were recorded.
Results: 36 contacts were referred for screening, of whom 29 were screened, 28 with both QFN and Mantoux. 20 patients had ⩾8 hours exposure, with 8 (40%) having a positive QFN test compared with 3 (15%) a positive Mantoux test. Of the 9 patients with <8 hours exposure 1 (11.1%) had a positive QFN test, 2 (22.2%) having positive Mantoux tests. 60% of positive Mantoux tests were known to have had BCG vaccination, with 22.2% of QFN positive patients having had BCG.

QFN and TST results stratified by 8 hour exposure

	Duration of exposure	
	<8 hours	>8 hours
QFN +ve	1	8
QFN −ve	8	12
TST +ve	2	3
TST −ve	7	17

Conclusion: QUANTIFERON-TB GOLD testing in a hospital associated outbreak of TB correlates well with ⩾8 hour duration of exposure, in agreement with WHO guidelines. Bigger prospective studies, comparing IFN-γ assays with Tuberculin skin testing, are needed to confirm this finding.

O476 Larger scale transmission of and isoniazid-resistant/rifampicin intermediate-resistant *Mycobacterium tuberculosis* strain

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Objectives: Nation-wide surveillance on transmission and resistance of *Mycobacterium tuberculosis* in the Netherlands has functioned since 1993. Presumably due to a lower transmissibility and a well-organised tuberculosis control, transmission of multi-drug resistant tuberculosis (MDR-TB) was so far limited to single secondary cases. However, a MDR strain with an unusually low level of rifampicin resistance (MIC 1–2 mg/l) was transmitted from a single source to nine persons, of whom three developed active disease so far.
In the current study we assessed the molecular basis of the INH and rifampicin resistance of the outbreak strain and its implications for transmissibility and therapy.
Methods: The katG and rpoB gene of the MDR strain were sequenced and the minimum inhibition concentration (MIC) to INH and rifampicin was determined. DNA fingerprinting of *M. tuberculosis* isolates and conventional contact tracing was performed to investigate the spread of the MDR strain.
Results: The respective outbreak strain had a Ser315Thr mutation in the katG gene (k315). INH resistant strains with k315 mutation were previously found to maintain a higher transmissibility than other INH resistant strains. An Asp516Tyr mutation was found in the rpoB gene (r516). Phenotypically, most of the outbreak isolates were interpreted resistant, but a part of the isolates were read as susceptible, which confused the therapy guidance and the surveillance of MDR-TB.
Conclusion: The relatively high rate of transmission of this k315/r516 variant may be related to evolutionary development of *M. tuberculosis* to maintain its transmissibility despite the adaptation to withstand the therapy by our most important anti-mycobacterial drugs; INH and rifampicin. More studies are needed to investigate the contribution of k315/r516 MDR strains to transmission of MDR-TB.
The consequences of the unusual low level of rifampicin resistance for therapy guidance and surveillance will be determined. It is considered to introduce the term "intermediate susceptibility" to indicate this level of rifampicin resistance.

O477 Evaluation of a new version of the "RT-TB" triplex real-time PCR assay for the rapid diagnosis of *Mycobacterium tuberculosis* in clinical samples

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Objectives: The "Real Time TB" assay (RT-TB) is a new multiplex real time PCR assay that allows the rapid detection and differentiation of the *M. tuberculosis* complex from other mycobacteria. The assay targets the IS6110 element and the RD9 specific region that

differentiate *M. tuberculosis* (IS6110 positive – RD9 positive) from the other mycobacterial species included in the *M. tuberculosis* complex (IS6110 positive – RD9 negative). We recently evaluated the first version of the RT-TB kit, demonstrating that the assay is sensitive, particularly in smear negative–culture positive clinical samples. Here, we report on the evaluation of a new version of the RT-TB assay in which the labelling of the IS6110 probe has been modified in order to improve its stability and detection level.

Methods: The new RT-TB BioRad kit includes an IS6110-BRD04 probe (instead of IS6110-Tamra in the previous version) that increases by 10-fold the sensitivity of detection of the IS6110 amplicons. Fifty-two clinical samples collected in routine (sputa, bronchoalveolar and gastric lavages) were included. DNA preparations and RT PCR reactions were carried out according to the manufacturer's instructions. A new specific software developed by the manufacturer was used for the automated analysis of the RT-TB amplification results.

Results: In the present study, 2 samples were found to contain inhibitors (as indicated by the negative amplification signals of the corresponding internal controls). The 8 smear positive samples included in the study were all found to be IS6110-POS and RD9-POS. Interestingly, 4 of them showed <1 acid fast bacilli per field on microscopic examination. The good sensitivity of the test was confirmed by 2 smear negative samples which were both found to be IS6110-POS and RD9-POS by the RT-TB assay. These two samples were confirmed to be positive for *M. tuberculosis* by the Roche Amplicor assay. Finally, the 40 remaining smear-negative samples were all found to be negative for IS6110 and RD9, suggesting that the increased sensitivity of the new IS6110-BRD04 probe does not impair the specificity of the test.

Conclusion: The results obtained for the first evaluation of the new version of the RT-TB kit suggest that the assay is sensitive and specific and may be very promising for the detection of *M. tuberculosis* in clinical samples containing few bacilli. Further experiments are in progress to confirm these preliminary data.

O478 Rapid detection of rifampin resistance mutations in clinical isolates of *Mycobacterium tuberculosis* by Dot-Blot hybridisation assay

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Objectives: A Dot-Blot hybridisation assay that detects all mutations occurring in the *M. tuberculosis* rpoB hot-spot region is being developed. The assay uses five probes, capable of binding to a different target segment within the rpoB hot-spot region of the wild-type *M. tuberculosis* genome. The present study is a preliminary investigation to assess the suitability of the assay for detection of resistance mutations in rpoB in clinical isolates of *M. tuberculosis* from Delhi, India.

Methods: Susceptibility testing of 142 isolates of *M. tuberculosis* was carried out by proportion method and confirmed by BACTEC 460TB system. Dot-Blot assay was performed on 106 isolates with two of the probes hybridising to the wild-type sequence of *M. tuberculosis*, from codons 522 to 527 (Probe D) and 528 to 533 (Probe E). Absence of hybridisation with any of the probes in the assay when a mutation was present indicated Rifampicin resistance, a surrogate marker for Multidrug-resistant *M. tuberculosis*.

Results: Susceptibility testing of 142 isolates revealed isoniazid resistance in 53.5%, rifampicin resistance in 41%, streptomycin resistance in 53% and ethambutol resistance in 37% of strains. Forty-five strains (32%) were multidrug resistant. Further analysis of the data showed that 96.4% of rifampicin-resistant strains and 100% of ethambutol-resistant strains had co resistance to one or the other antituberculous drug. It was also interesting to note that 77% of the rifampicin-resistant strains were multidrug resistant, while the corresponding figure for ethambutol-resistant strains was 87%.

Dot-Blot hybridisation assay with probes D and E was carried out on 106 isolates of *M. tuberculosis*, of which 43 were resistant to rifampicin. Of the rifampicin-resistant isolates tested, 15 did not hybridise with probe E, indicating a mutation at this site. The results of 10 strains were confirmed by sequencing when a mutation was detected in 9 strains at codon 531 and in one of them at codon 533. The remaining 28 rifampicin-resistant strains, which hybridised with probe E, may have a mutation at a site other than that complimentary to probe E. Of these, 10 hybridised with probe D, indicating a mutation at the site complimentary to probe D (codons 522 to 527). Of the 63 rifampicin-susceptible isolates, 62 (98%) hybridised with probes D and E, indicating a wild-type sequence.

Conclusions: The Dot-Blot assay was found to be a sensitive and specific assay.

O479 Rapid detection of multidrug-resistant and heteroresistant tuberculosis in one day using the new molecular-biological test Genotype MTBDR^{TM}

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MDR-TB is defined by resistance of the respective pathogen (*Mycobacterium tuberculosis* complex, MBTC) against INH and rifampin RMP. Mortality of MDR-TB is double compared to sensitive TB. Phenotypic drug-susceptibility-testing (DST) lasts up to 8 weeks. Following the DOTS and waiting for DST results, MDR-TB patients are miss-treated with INH and RMP. A new resistance test, Genotype MTBDR^{TM} (G-MDR), has been released, detecting MDR-TB. The test is based on PCR and hybridisation.

For its validation, 64 strains have been characterised by threefold DST on solid and liquid media. 100% of RMP resistances (n = 31) and 90% of INH resistances (n = 49) were detected by G-MDR. Specifity was 100%. 112 TB isolates from highly endemic countries were analysed: 31 from Bolivia, 58 from Nepal and 23 from Ukraine. 93% of RMP-resistances were detected with G-MDR. The sensitivity for detection of INH resistance increased with the prevalence of MDR-TB in the respective countries from 64% in Nepal, 74% in Bolivia, 83% in Usbekistan, to 86% in the Ukraine. All G-MDR assays for Nepalese strains were performed in the National Reference TB-Laboratory in Katmandu, demonstrating that the test is robust enough to be applied in developing countries. Finally, the test was applied to 35 sputum specimen from Usbekistan, which were all positive in microscopy. All MDR cases have been detected. Additionally, heteroresistance to INH and RMP could be visualised in nine cases.

Genotype MTBDR^{TM} is a fast, easy to perform and robust test, applicable in highly endemic countries. Its sensitivity for the detection of RMP resistance is 100%, for INH up to 90% depending on the MDR prevalence. It is an excellent tool for the detection of heteroresistance. The test can be performed from microscopically positive sputum, reducing the DST time to zero days.

Molecular diagnostics

O480 Characterisation of *Neisseria meningitidis* B causing invasive disease in the Czech Republic

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Objectives: Invasive meningococcal disease (IMD) caused by *Neisseria meningitidis* B is endemic in the Czech Republic and its long-term incidence is stable (0.5/100,000 population), reaching peaks in the youngest age groups (up to 22.6/100,000 for 0–11 months olds and 2.7/100,000 for 1–4 years olds). The aim of this study was to characterise *N. meningitidis* B isolates from IMD and to assess the coverage of vaccines against *N. meningitidis* B.

Methods: The total number of *N. meningitidis* B isolated from IMD in the Czech Republic in the period 1993–2006 (Nov. 8) was 474. In all isolates serogrouping and sero/subtyping was performed. Subtyping by Whole Cell ELISA (WCE) was replaced by PorA sequencing recently (http://neisseria.org/nm/typing/). Sequence types (STs) were identified by multilocus sequence typing (MLST) in accordance with the MLST

website (http://pubmlst.org/neisseria/). The number of *N. meningitidis* B IMD isolates investigated by MLST was 366.

Results: Sero/subtyping showed high heterogeneity: among 474 isolates, 88 phenotypes were found. The most frequent phenotype was B:4:P1.15 (15.4%), followed by B:15:P1.7,16 (8.2%) and B:15:P1.5 (5.7%). 15.4% of isolates were not typeable/subtypeable by WCE. PorA sequencing of these isolates further identified their heterogeneity. MLST confirmed this high heterogeneity: 142 STs, belonging to 16 clonal complexes, which represent 79.2% of isolates. There were three prevailing complexes: ST-18 complex (19.1%), ST-32 complex (18.8%) and ST-41/44 complex (16.9%). The Czech *N. meningitidis* B population is different compared to western Europe: 109 of the STs (76.8% of STs) and one clonal complex (ST-292 complex) were described for the first time in the Czech *N. meningitidis* B isolates. Coverage of sero/subtypes by currently being developped vaccines against *N. meningitidis* B is low (maximum 44.5% for nine-valent meningococcal B PorA vaccine).

Conclusion: Detailed characterisation of *N. meningitidis* B isolates from IMD in the Czech Republic in the period 1993–2006 showed their high heterogeneity and low coverage by currently available vaccines against *N. meningitidis* B.

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O481 Detection by DHPLC of 23S rDNA mutations responsible for clarithromycin resistance in *Helicobacter pylori*

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Objectives: Resistance to clarithromycin is the leading cause of decrease in *H. pylori* eradication. Since 23S rRNA mutations correlated with in vitro and in vivo clarithromycin resistance, routine detection of these mutations will be helpful to adapt the antibiotic treatment.

Methods: A total of 123 clinical strains of *H. pylori* consecutively isolated between 2004 and 2005 in our hospital have been studied. Clarithromycin MICs were determined by both the agar dilution and E-test method. A 147-bp fragment, encompassing the nucleotides involved in clarithromycin resistance at positions 2146 and 2147 in 23S rDNA, were amplified and analysed by denaturing high pressure liquid chromatography (WAVE® systems, Transgenomic). The DHPLC analysis was done on PCR products after mixing PCR product from the tested strain with that from a wild-type susceptible reference strain in order to create heteroduplexes. The 23S rDNA was also studied by direct sequencing for all clarithromycin-resistant strains and a large part of susceptible strains.

Results: DHPLC profiles were identical to that of the wild-type strain for 85/123 (69%) strains, which were all susceptible to clarithromycin (MICs from 0.06 to 0.5 mg/l) except two strains for which a mix of susceptible and resistant (MIC of 8 and 256 mg/l) strains was observed. DHPLC profiles were different from that of the wild-type strain for 35 strains (28.5%) which were all resistant to clarithromycin (MICs from 6 to >256 mg/l) except one strain for which again a mix of susceptible (MIC of 0.05 mg/l) and resistant strains was observed. For 3 (2.5%) strains, we obtained undetermined DHPLC profiles, i. e. neither a profile identical to a wild-type strain or a mutated strain. Different DHPLC retention profiles were observed for the two main mutations, A2146G (n = 3) and A2147G (n = 32). No strain harbouring the A2146C was observed. We also evaluated the minimal proportion of resistant strains with a mutated allele that can be detected. This was about 25% with DHPLC as with sequencing methods.

Conclusion: DHPLC method is a reliable method to detect 23S rDNA mutations predictive of clarithromycin resistance.

O482 Comparison of a molecular screening method with traditional culture for the detection of *Salmonella* spp. and *Campylobacter jejuni* in faeces

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Objective: *Salmonella* spp. and *Campylobacter jejuni* are the major causes of bacterial gastro-enteritis in the Netherlands. Conventional diagnosis is based on detection of both species in faeces by traditional culturing which usually takes several days. We developed a sensitive molecular screening method (MSM) for the detection of both species which decreases the turn-around time significantly. This study describes the comparison of this real-time PCR based screening method with routine culture for the detection of *Salmonella* spp. and *C. jejuni* in faeces.

Methods: A total number of 2,067 stool samples were analysed at our laboratory. Routine culture was performed on faecal samples and consisted of enrichment, selective culture and phenotypic identification. The molecular method consisted of a semi-automatic DNA extraction in combination with real-time PCR assays for *Salmonella* spp. and *C. jejuni*. PCR positive samples as well as samples which demonstrated PCR inhibition were cultured afterwards, consisting of the identical procedure as described for routine culture. Also, data regarding time to generate final results were collected for the MSM.

Results: A total number of 2,055 samples were included for validation of *Salmonella* spp. The detection of *Salmonella* spp. improved by 15% with molecular screening; sensitivity was 100% and specificity 99%. For *C. jejuni* 2,009 samples were included and detection improved by 25%; sensitivity was 97% and specificity 75%. PCR inhibition was observed in less than 1.9% of all samples. The time to generate final results was less than 24 hours for all PCR negative samples (with exception of the inhibited samples), and in comparison to traditional culture there was no delay observed in generating results of culture confirmed PCR positive samples.

Conclusions:

1. The MSM has a great potential for rapid detection of *Salmonella* spp. and *C. jejuni* in faeces.
2. Time to generate final results for negative samples was reduced dramatically to less than 24 hours.
3. The detection of *Salmonella* spp. and *C. jejuni* will improve considerably with molecular screening.
4. Automation of the extraction and detection procedures will further speed up the process and improve standardisation of the molecular screening procedure.

O483 Staphylococci speciation and Panton-Valentine leukocidin detection by matrix-assisted laser desorption ionisation time-of-flight mass spectrometry

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Objectives: A rapid proteome analysis has proven useful for microbial identification. We assessed a matrix-assisted laser desorption ionisation time-of-flight (MALDI-TOF) mass spectrometry for the rapid identification and differentiation of staphylococcal species as well as the detection of Panton-Valentine Leukocidin (PVL) toxin from clinical staphylococcal isolates.

Methods: Staphylococcal clinical isolates recovered from blood culture were included in the study. Phenotypic identification and differentiation between *Staphylococcus aureus* and coagulase-negative staphylococci (CoNS) were determined by colony morphology, catalase, and a Staphaurex latex agglutination. CoNS speciation was performed by using a bioMérieux/VITEK API STAPH system. Methicillin resistance in *S. aureus* strains was confirmed by disk diffusion. The PVL gene was detected by a real-time TaqMan PCR assay.

Results: Among a total of 115 staphylococcal isolates, 41 (35.7%), 44 (38.3%), and 30 (26.1%) were identified as CoNS, methicillin-

resistant *Staphylococcus aureus* (MRSA), and methicillin-susceptible *S. aureus* (MSSA), respectively, by the MALDI-TOF with correct identification rates of 100%, 97.7% and 93.3%, in comparison with those identified by phenotypic methods. The agreement rate was 75.6% with those determined by the API STAPH when these CoNS isolates were identified into species level by the MALDI-TOF. Seven PVL-positive MRSA isolates were correctly recognized by the MALDI-TOF with 100% accordance with the results determined by real-time PCR. The whole process, from the sample preparation to result analysis, can be completed between 1.5 and 2.5 hours, which greatly shortens the time usually needed for current phenotypic identification.
Conclusion: MALDI-TOF mass spectrometry provides a rapid and relevant system for clinical identification of staphylococci. Detecting PVL protein directly from clinical isolates provides a bacterial identification system that is desirable in clinical diagnostic services.

O484 Development of a multiplex PCR assay for enterotoxigenic *Bacteroides fragilis* and detection of this emerging pathogen in cases of community-acquired diarrhoea in the UK

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Objectives: *Bacteroides fragilis* is the Gram-negative anaerobe isolated most often from human infections. Some *B. fragilis* strains produce a 20 kDa enterotoxin (BFT). Enterotoxigenic *B. fragilis* (ETBF) have been isolated from various diarrhoeic animal species and epidemiological studies worldwide note a significant correlation between ETBF and human diarrhoeal disease, especially in children. The prevalence of ETBF in gastrointestinal diseases in the UK, however, has not been investigated, partly due to the lack of a simple identification test that can be used in diagnostic laboratories. The aim of this study was to develop a sensitive and specific multiplex PCR assay for the detection of ETBF directly from faeces, and to investigate if ETBF are associated with cases of community-acquired diarrhoea in the UK.
Methods: Primers were designed from published enterotoxin sequences to amplify 416 bp of bft, and multiplexed with primers amplifying 293 bp of nanH (+ve control for presence of *B. fragilis*). The assay was validated against 136 strains from 52 species. DNA was extracted from stools using the QIAamp DNA stool mini kit (Qiagen). Spiking experiments were used to determine sensitivity of the assay. Stool was obtained from 193 cases of community-acquired diarrhoea, selected on the basis that no other bacterial pathogen had been identified. Where bft was detected in DNA extracts, a second novel multiplex PCR assay was used to determine which of the 3 bft isoforms was present.
Results: The PCR assay for ETBF was found to be 100% specific and had a detection limit of 10^4 cfu/g stool. 13% (25/193) of the diarrhoea samples gave a positive result for bft and nanH. The predominant isoform in the ETBF +ve samples was bft-1 (n = 20, 80%). Three samples had bft-2 and 1 had bft-3. A diarrhoeal sample from a 1-yr old male yielded PCR amplimers for both bft-1 and bft-2 suggesting carriage of at least 2 different ETBF strains.
Conclusion: The multiplex PCR assay was highly specific for ETBF and allowed detection without the need for culture. This is the first report of ETBF in community-acquired diarrhoea in the UK. The distribution of isoforms in the clinical samples was similar to earlier reports from Europe, but the occurrence of 2 different bft isoforms in a faecal sample has not been described before. The finding of ETBF in a high proportion (13%) of samples for which there was no other bacterial explanation for the diarrhoea merits further investigation of this pathogen.

O485 Recently identified viruses contribute significantly to acute respiratory infections in children

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Objectives: More than half of all episodes of acute respiratory infection (ARI) have none of the pathogen identified in paediatric as well as adult populations. It is likely that the prevalence of viral infection is underestimated because of the large number of respiratory viruses involved in respiratory tract infection. A number of novel respiratory pathogens had been identified since 2001, i.e. human metapneumovirus (hMPV), coronaviruses NL63 and HKU1, human bocavirus (hBoV); they are not currently diagnosed but could contribute significantly to the burden of ARI. A molecular approach was undertaken to detect nearly all respiratory viruses, including hMPV, NL63, HKU1 and hBoV in hospitalised children suffering from acute respiratory tract infection.
Methods: Most respiratory viruses were detected prospectively in nasal washes from children hospitalised for ARI in a paediatric department at the University "La Sapienza" hospital of Rome. HKU1 and bocavirus were searched for retrospectively, on frozen aliquots of samples. Reverse transcription-PCR assays followed by sequencing of the amplified fragments were undertaken to detect fourteen respiratory viruses: influenza A and B, RSV, hCoV OC43 and 229E, adenovirus, rhinovirus, parainfluenza viruses 1–3, hMPV, NL63, HKU1 and hBoV.
Results: In 103/227 children (45.4%), at least one viral pathogen was identified; 86/227 (37.9%) had an infection with one of the virus investigated; 17/227 (7.5%) had a dual infection, with a total of 120 viruses identified. The most common agent was RSV, followed by rhinovirus and parainfluenza virus 3. Overall, hMPV infections represented about 8% of all viral illness. One NL63 case was detected; no positive to HKU1 was found. Bocaviruses were detected in 12 cases (11% of all virus positive cases), half of which in co-infections. Almost all patients who had hBoV as the sole pathogen had pneumonia; in addition, it was detected in one children hospitalised for bronchiolitis.
Conclusions: Detection of the recently characterised metapneumovirus, coronaviruses NL63 and HKU1, and bocavirus contributed a significant proportion (17.5%) of all positive samples.
This study is a confirming report of NL63 and hBoV circulation in Italy, reported in late 2006. Interestingly, bocavirus was a frequently detected respiratory agent and was associated with clinically important illnesses.

O486 Implementation of a real-time RT-PCR assay to improve diagnostics of dengue virus infections

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Objectives: Dengue viruses (DENV) are the most common insect-borne viral pathogens diagnosed in travellers returning from tropical zones. The symptoms caused by DENV range from self-limited, flu-like illness, to life-threatening haemorrhagic fever. DENV are immunologically grouped in four serotypes and secondary infection by a different serotype is presumably one of the factors favouring the haemorrhagic presentation. Routine diagnostics rely on the detection of DENV-specific IgM and IgG. However, because of the low antibody titres in the early phase of disease, serology is prone to false negative results. By allowing detection of the virus in this early phase, RT-PCR can close the diagnostic gap. The objective of this study was to improve the quality of DENV diagnostics at the Zentrum für Infektionsdiagnostik (ZID, Basel) by real-time quantitative RT-PCR, for early detection and molecular typing.
Methods: Serological testing of patient samples was performed by using a rapid commercial assay detecting DENV specific IgM and IgG. A quantitative pan-dengue real-time RT-PCR (PAND PCR) protocol was used to detect DENV independently of the subtype. Classification into the four DENV subtypes was achieved using a serotype specific multiplex real-time RT-PCR protocol. Commercial kits for RNA extraction (QIAamp®, Qiagen) and one-step RT-PCR (iScript®, Biorad) were used. Overall, 98 serum samples were analysed with both methods: subset A was a retrospective panel of 25 IgM positive serum samples; subset B consisted of 73 serum samples (22 IgM positive, 51 IgM negative) collected prospectively at the ZID between April and November 2006.
Results: Of 47 IgM positive samples, 17 (36%) were positive by PAND PCR (subset A: 4 from 25, 28%; subset B: 13 from 22, 59%). Of 51 IgM negative samples (all subset B), 6 were positive by PAND PCR (12%). The detected viral load ranged from 255 copies/mL to

7.13^7 copies/mL (median 1.33^5 copies/mL). Two PAND PCR positive and 4 negative samples were sent to a reference laboratory for confirmation. In one case, the result was discordant. Of 16 samples tested with the serotype specific RT-PCR, 13 (81%) could be attributed to a subtype: 7 to subtype I, 2 to subtype II, 2 to subtype III and 2 to subtype IV.

Conclusion: Our results show that a combination of serological and RT-PCR are required for rapid and reliable dengue virus diagnostics at all stages of infection.

O487 RespiFinder-kit: simultaneous detection of 15 atypical viruses commonly involved in respiratory tract infections

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Objective: Acute respiratory tract infection is the most widespread type of acute infection in adults and children. The number of pathogens involved is numerous. The objective is the development of a multiparameter assay which enables the detection of all pathogens commonly involved in respiratory tract infections. The RespiFinder test currently involves influenza A, B and H5N1 virus, respiratory syncytial virus A and B, parainfluenza virus type 1, 2, 3 and 4, coronavirus 229E, OC43 and NL63, rhinovirus, human metapneumovirus and adenovirus (subgenera A, B, C, D, E and F). The aim was to obtain the same specificity and sensitivity as singleplex QPCR and to complete detection and differentiation within 6 hours.

Method: The RespiFinder-kit is based on a new multiplex PCR technology which enables simultaneous amplification of up to 40 fragments. Detection of a virus is dependant on two specific probes. These two probes hybridise adjacently to each other. After ligation of the specific probes, all targets are amplified with one universal primerset. The amplified fragments can be discriminated by size fractionation. A competitive internal RNA control is added to each sample allowing discrimination between a true negative sample and a negative sample due to a PCR failure. Prior to the multiplex amplification, viral RNA is converted into cDNA using an one step RT-PCR followed by a limited number of PCR cycles.

Results: The RespiFinder-kit was compared with singleplex QPCR assays as well as conventional culturing procedures. Sensitivity of the assay was compared with QPCR using serial dilutions of virus cultures. The same sensitivity was obtained with the RespiFinder-kit as with QPCR. Clinical samples were tested with all three approaches. This showed increased sensitivity of the two nucleic acid based tests over the conventional diagnostic procedures. The results with the RespiFinder-kit and the QPCR assay showed a high degree of correlation.

Conclusions: We showed that the RespiFinder-kit enables simultaneous detection of 15 viruses commonly involved in respiratory tract infections within 6 hours and with the same sensitivity and specificity as singleplex QPCR reactions.

O488 High prevalence of *Legionella pneumophila* in severe community-acquired pneumonia, as determined by a commercially available PCR assay

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Objectives: *Legionella pneumophila* is one of the main causative agents of severe community acquired pneumonia (CAP). When legionellosis occurs, it can be fatal if diagnosis and treatment are not promptly established, particularly in elderly and immunocompromised patients. Therefore, rapid detection and diagnostic methods are needed to improve the outcome of infected patients and to reduce unnecessary antibiotic therapy. The main goals of this work were:

- To assess the use of a new PCR-based method to support *L. pneumophila* diagnosis in patients with severe CAP, as compared to current laboratory methods.
- To determine the prevalence of *L. pneumophila* in severe CAP, requiring admission in intensive care unit (ICU).

Methods: A total of 95 clinical specimens (BAL, blood and urine) from 39 patients admitted in the intensive care unit (ICU) with severe CAP, were collected from December 2004 to October 2006. The presence of *Legionella pneumophila* was assayed simultaneously by the antigen urine test (Binax®), by microbiological culture in selective charcoal medium and by conventional PCR using a commercially available kit (Legiofast, Microbial SL).

Results: PCR analysis of *Legionella pneumophila* was positive in 21 patients (56.4%) whereas only 9 (23.1%) resulted positive when the enzyme immunoassay technique was used. No positive results were obtained by plate culture. No false negatives were obtained with the PCR kit. Moreover, all positives with Legiofast were in accordance with clinical parameters of *Legionella* infection.

Conclusions: The PCR assay used in this work enables a rapid and sensitive diagnosis using as low as 200 µl of urine. Prevalence of *L. pneumophila* is significantly higher when analysed by PCR as compared to the urine antigen test ($P = 0.01$), suggesting that the prevalence of this pathogen in severe CAP is higher than suspected so far. Since urine antigen test is restricted to serogroup 1 of *L. pneumophila*, the fact that the PCR kit detects serogroups 1 to 15 may help to explain this difference. The use of this PCR as a complement of conventional techniques is recommended to improve the detection of *L. pneumophila* in clinical laboratories.

O489 Cost-effective method for differentiation between *Salmonella* species and other members of the Enterobacteriacae referred to the national *Salmonella* reference laboratory in England and Wales

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Objectives: Traditional methods for isolation and identification of *Salmonella* species require a series of biochemical and serological tests. Our purpose was to determine the cost effectiveness of incorporating molecular testing for differentiation between *S. enterica*, *S. bongori* and other members of the Enterobacteriacae. The latter are commonly referred to our laboratory for confirmation as "probable salmonellas" or *Salmonella* cultures are received contaminated with other organisms. These require selective isolation prior to identification. This in turn requires time and expense, including special media, additional exposure to microbes and more technologist time to perform subcultures. As many laboratories face budgetary constraints including loss of personnel, we propose a simple, cost-effective method of confirming *Salmonella* sp. prior to typing.

Method: We tested over 350 known Enterobacteriacae with a simple PCR assay. On the basis of their initial biochemistry, e.g. atypical results, we also tested 250 "probable salmonella" isolates referred to us for typing. Oligonucleotide primers were prepared according to the sequence of the chromosomal invA gene and the PCR assay was performed from a boiled cell template. An internal amplification control (IAC) was included to indicate possible PCR inhibitors. The trial now aims to evaluate 6-month resource use and costs of both PCR and traditional methods.

Results: The primers were specific as no amplification products were obtained for over 150 isolates of non-*Salmonella* Enterobacteriacae covering 20 species. In contrast, we were able to detect the target amplicon from a wide range of *Salmonella* strains and sub-groups. All 96 serovars (211 isolates) tested were shown to carry the invA gene. Most of the "probable *Salmonella* spp." did not produce a PCR product. Further work confirmed them as non-salmonellas. For the isolates that did demonstrate the presence of the invA gene, further testing found that these were salmonella cultures contaminated with other organisms. Current cost savings already demonstrated by using the PCR assay were largely attributable to the time taken by the technologist to perform all the traditional tests before producing a final customer report.

Conclusion: This assay provides a cost-effective rapid means of distinguishing between routine specimens that have been misidentified as *Salmonella* strains and those that do actually require further selection and typing.

Beta-lactam resistance

O490 blaOXA-58 gene is overexpressed and contributes to the carbapenem heteroresistance in *Acinetobacter baumannii*

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Objectives: To characterise the carbapenem heteroresistant phenotype in *Acinetobacter baumannii* strains and elucidate the underlying molecular mechanism.
Methods: The study included five *A. baumannii* strains with subcolonies grown within the carbanepem inhibition halo in disc diffusion assay. Susceptibility status was tested by the disc diffusion assay and carbapenem MICs were determined by Etest and Etest MBL. Genotyping was performed by ERIC-PCR. Heteroresistant subpopulations were recovered by population analysis and subsequently tested by Etest and disc diffusion. Expression of genes blaOXA-51, blaOXA-58 and ampC was quantified by Real-Time PCR. Outer membrane proteins were analysed by SDS-PAGE. The presence of ISAba1 and ISAba3 relatively to blaOXA-51 and blaOXA-58 was tested by PCR mapping. OXA-58 inhibition experiments with 200mM NaCl were performed to check the contribution of blaOXA-58 to the carbapenem heteroresistance.
Results: All strains were resistant to all tested antimicrobials except carbapenems and colistin and negative for metallo-β-lactamase. ERIC-PCR showed four genotypic profiles. Etest MICs for carbapenems ranged from 0.38 to 32 mg/L for imipenem and 0.38 to 6 mg/L for meropenem. Population analysis showed imipenem MIC of heteroresistant populations from 4 to 128 mg/L while meropenem MIC from 0.50 to 16 mg/L with respective frequencies ranging from 4×10^{-6} to 9×10^{-5} and from $2\times10^{—6}$ to 2×10^{-4}. All strains showed heteroresistant subpopulations with lower carbapenem susceptibility. Four of them were unstable after subcultures in drug-free medium, while one produced stable carbapenem heteroresistant subpopulations. Real-Time PCR showed increased expression of blaOXA-58 in both imipenem and meropenem heteroresistant populations, whereas genes blaOXA-51 and ampC were unchanged. OMP analysis showed no particular differences. One isolate was positive for ISAba1 which was unrelated to blaOXA-51 and two isolates were positive for ISAba3, adjacent to blaOXA-58 allele. OXA-58 inhibition experiments using 200mM NaCl showed approximately 4-fold reduction of carbapenem MIC in the heteroresistant subpopulations.
Conclusions: Gene blaOXA-58 appears to elevate carbapenem MICs in carbepenem heteroresistant *A. baumannii* strains. Thus, laboratory susceptibility assays may need to be adjusted in order to detect the heteroresistant subpopulations.

O491 *Pseudomonas aeruginosa* with a novel blaVIM-4/blaP1b and a second class-1 integron, efflux pumps overexpression and repressed porin OprD

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Objectives: To investigate the resistance mechanisms in a multi-drug resistant *Pseudomonas aeruginosa* isolate which was positive for metallo-β-lactamase (MBL).
Methods: Strain *P. aeruginosa* 140 was studied. Identification and susceptibility testing to antipseudomonal antimicrobials was performed by the VITEK 2 automated system. MICs of imipenem, meropenem, ceftazidime, aztreonam, gentamicin and piperacillin/tazobactam were determined by agar dilution. Etest MBL and the imipenem–EDTA double disc synergy test (DDST) were done to detect MBL production. PCR for gene blaVIM and conserved segments of class-1 integrons was performed. Mating experiments were performed by mixed broth mating using *P. aeruginosa* PU21 (rif^r) as recipient and plasmid isolation with alkaline lysis. Quantitative real-time reverse transcription-PCR (QRT-PCR) was performed in order to detect transcripts of genes mexB, mexY and ampC. Relative expression levels in strain 140 were compared against those in a susceptible isolate. Outer membrane proteins (OMPs) were analysed by SDS-PAGE.
Results: Isolate PA140 was resistant to all tested antimicrobials except aztreonam. Agar dilution MIC of imipenem was 512 and that of meropenem 128 mg/L, while MICs of both gentamicin and piperacillin/tazobactam were >512 mg/L. MICs of ceftazidime and aztreonam were 32 and 8 mg/L, respectively. Etest MBL exhibited marked decrease in MICs of imipenem compared to imipenem-EDTA (256 and 1.5 mg/L respectively) and DDST was positive. PCR revealed two class-1 integrons. The MBL-associated integron was sequentially consisted of genes blaVIM-4 and blaP1b coding for β-lactamases VIM-4 and PSE-1/CARB-2 respectively. The second integron comprised sequentially of genes aac(6′)-Ib and blaOXA-35. Plasmid extraction did not reveal any visible plasmids and no transcojugants were produced by mating experiments. QRT-PCR revealed overexpression of genes mexB and mexY as well as a fairly detectable expression of gene ampC in strain PA140 relatively to the susceptible strain. Also, OMP analysis revealed a lower intensity of a protein band of MW $\approx$ 46 kD in strain 140, consistent with OprD.
Conclusions: A *P. aeruginosa* clinical isolate harboured a novel class-1 integron with two (blaVIM-4 and blaP1b) β-lactamase gene cassettes and a second integron. The isolate was highly carbapenem- and multidrug-resistant, had reduced production of porin oprD and overexpressed efflux pumps MexAB-OprM and MexXY-OprM.

O492 VIM-2 metallo-β-lactamases genes found in *Pseudomonas aeruginosa* and *Acinetobacter* spp. from Russia and associated with unusual integrons

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Objectives: To determine the mechanism of resistance of eight multi-resistant clinical isolates collected by the National Research Centre of Antibiotics, Moscow, Russia in 2005.
Methods: Strains were identified using an API 20 NE kit. The MICs were determined using Etest ESBL or MBL strips (AB biodisk, Solna, Sweden) and a wide range of antimicrobial test discs. Isolates positive by Etest MBL strips were further investigated for resistance determinants by PCR using blaVIM, blaIMP, blaSPM-1, blaGIM, blaSIM-1 and class-1 integron specific primers, as well as primers designed to the transposition genes of Tn5090. Amplicons were sequenced by using custom designed primers using standard methods.
Results: Six strains were identified as *P. aeruginosa* and one as *Acinetobacter* spp. All strains were resistant to imipenem >256mg/L which reduced in the presence of EDTA from 1 to 8 mg/L (MBL Etest strip). All isolates were multi-resistant and only sensitive to colistin. Five *P. aeruginosa* isolates harboured the blaVIM-2 gene cassette in a class-1 integron containing the gene cassette array: aacA7, blaVIM-2, dhfrB5 and aacA5. The structure was very unusual as it did not contain a 3′ conserved sequence (3′CS). However, it did contain Tn5090 transposition genes, absent from most commonly found integron structures. The integron array was identical to those found in *P. aeruginosa* strains harbouring the blaVIM-2 gene recently characterised from the USA and Norway, and very similar to one recently characterised from India. One strain of *P. aeruginosa* and an isolate of *Acinetobacter* spp. also contained the blaVIM-2 gene in a normal class-1 integrons as gene cassettes in the first position that also contained an OXA-2 gene cassette.
Conclusion: The absence of a 3′CS and the presence of the tniC transposition gene suggests that the integron containing the VIM-2 allele in most of the *P. aeruginosa* isolates predates the formation of both the 3′CS and a deletion event that immobilised the Tn5090 transposon that carries the class-1 integron. The geographic widespread location of this particular integron structure – including blaVIM-2, suggests recent worldwide dissemination.

O493 VIM-2 metallo-β-lactamase emerges in *Pseudomonas aeruginosa* isolated from India

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Objectives: To determine the mechanism of high-level carbapenem resistance in *P. aeruginosa*, isolated from a bronchoalveolar lavage of a 60 year old male suffering from ventilator-associated pneumonia in 2003, from Chennai, India.
Methods: The MICs to various antimicrobials were assessed by Etest. The presence of a carbapenemase was determined by hydrolysis of imipenem with and without EDTA (25mM). A MBL-like phenotype was confirmed by the MBL Etest strip (AB biodisk, Solna). PCR was performed using blaVIM, blaIMP, blaSPM-1 blaGIM blaSIM and class-1 integron specific primers, as well as primers designed to the transposition genes of Tn5090. Amplicons were sequenced by using custom designed primers followed by sequencing using standard methods. The genetic location of the blaMBL resistance gene was investigated by conjugation experiments with *P. aeruginosa* PA01, Southern hybridisation and plasmid isolation.
Results: The *P. aeruginosa* isolate harboured a class-1 integron containing an array of aacA7, blaVIM-2, dhfrB5 and aacC6-II gene cassettes. The 59 base element of the aacC6-II gene cassette was truncated by the insertion of ISPa21. The class-1 integron structure was very unusual as it did not contain a 3′ Conserved Sequence (3′CS) consisting of fused qac and sul1 genes. However, it did contain Tn5090 transposition genes, absent from most commonly found class-1 integron structures. The integron array was very similar to a structure found in a *P. aeruginosa* strain harbouring the blaVIM-2 gene recently characterised from the USA. Conjugation and plasmid isolation experiments failed to transfer resistance to PA01; however, Southern hybridisation demonstrated that blaVIM-2 is carried on a plasmid. The concomitant loss of resistance to ceftazidime and the blaVIM-2 gene was observed after overnight growth at 37ºC confirming the hybridisation data.
Conclusion: This is the first description of *P. aeruginosa* strains harbouring blaVIM-2 from India. This further extends the geographic distribution of this multi-resistance conferring allele. The absence of a 3′CS and the presence of the tniC transposition gene suggests that this integron predates the formation of both the 3′CS and a deletion event that immobilised the Tn5090 transposon that carries the class-1 integron.

O494 Plasmid analysis and location of the OXA-40 carbapenemase gene in multidrug-resistant endemic clones of *Acinetobacter baumannii*

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Objective: To analyse the presence of plasmids and to demonstrate the relation of these structures with the bla OXA-40 carbapenemase.
Methods: Isolates belonging to endemic clones I and II were selected from the collection of isolates of *A. baumannii* obtained from a hospital in Northern Spain during the years 1999, 2002 and 2005. The representatives were three per year and clone including OXA-40 positive and negative isolates. Clonal relatedness was confirmed by amplification experiments with M13 and AP3 primers and PFGE with Apa I enzyme. Alkaline lysis method and commercial kits were used to detect the plasmid content. To locate bla OXA-40 Southern transfer hybridisation experiments were carried out using a specific DNA probe labelled with digoxigenin. Preliminary mapping of plasmid DNA was achieved with EcoRI, PstI and Hind III restriction enzymes.
Results: Plasmids of similar sizes were observed in all isolates belonging to both clones, frequently combined in groups of several structures. The size of the plasmids were 125, 112, 84, 32, 8, 4, 3.4 and 2.5 kb. Our results showed that the same plasmids were in both clones and that there were some structures which were always presented in groups. Concerning the blaOXA-40 location we could see different signals on different bands. Although some isolates showed a chromosomal positive signal, the majority of them also showed it on different plasmids. Although high diversity was found, it was the plasmid of 32 kb the main structure where the positive hybridisation signal was seen. Mapping of a representative isolate containing that plasmid showed different fragments were the blaOXA-40 gene could be located.
Conclusion: Although the predominant clones producing the OXA-40 carbapenemase in our environment are genetically unrelated they bore plasmids of the same size. Isolates from both clones bore plasmids from 2.5 to 125 kb frequently associated in groups. We could see the same plasmids in isolates obtained along the period of study which means that they are stable among the population. The predominant location of the blaOXA-40 gene was on plasmids.

O495 New VIM-2-like metallo-β-lactamases in *Pseudomonas aeruginosa* from Germany and Bulgaria

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Objectives: The incidence of *Pseudomonas aeruginosa* strains resistant to carbapenems due to the production of class-B metallo-β-lactamases (MBLs) is increasing world-wide. We analysed the β-lactamases of carbapenem-resistant *P. aeruginosa* strains from Germany and Bulgaria.
Methods: MICs were determined by the microdilution technique according to CLSI-guidelines. Double disc synergy test with EDTA and the Hodge-test were carried out. Oligonucleotides specific for various groups of MBLs were used to identify the bla gene. Assuming the bla genes to be part of an integron, primers binding to intI1 and qacEdelta1 were used for PCR and sequencing.
Results: *P. aeruginosa* 166301 from Munich had an MIC of 64 mg/L for meropenem and of 8 mg/L for imipenem. *P. aeruginosa* 9553 from Sofia had an MIC of 64 mg/mL for both drugs. Hydrolysis of carbapenems in the Hodge-test and an inhibitory effect of EDTA suggested the presence of MBLs. Sequencing revealed VIM-2-like β-lactamases in both strains. In comparison to VIM-2 the β-lactamase of *P. aeruginosa* 166301 showed one nucleotide exchange (C164T) causing an amino acid substitution from serine to leucine at position 54 while the enzyme of *P. aeruginosa* 9553 had another nucleotide exchange (A584T) resulting in an amino acid substitution from tyrosine to phenylalanine at position 218. Both VIM-genes were located on class-1 integrons. While blaVIM-2-like of *P. aeruginosa* 166301 was bracketed by two copies of the aminoglycoside acetyltransferase gene aac(6′)-Ib′, the integron of *P. aeruginosa* 9553 contained no further gene cassettes.
Conclusion: The detection of two novel VIM-2 variants with different amino acid substitutions and genetic environment in Germany and Bulgaria underlines ongoing multifocal evolution of the VIM-2 gene.

O496 Multiresistant *Klebsiella pneumoniae* from NYC hospitals contain β-lactamase KPC-2 or KPC-3 and porin changes

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Objectives: Multiply antibiotic resistant *K. pneumoniae* with high MICs to carbapenems have been endemic in several NYC hospitals since 1997. Recent evidence suggests that such strains have acquired genes encoding KPC carbapenemases and are spreading to other regions of the Eastern USA. Fourteen carbapenem-resistant strains isolated from the NY-Presbyterian Hospital in 2005 were examined for their susceptibility to antimicrobials, β-lactamase content and presence of mutations in ompk porin genes that would confer carbapenem resistance.
Methods: MICs were determined in cation adjusted Mueller–Hinton medium using a microtiter plate method with a final test volume of 200 μL. Bla and ompk genes were amplified by PCR using primers and control strains previously characterised. Pulsed field gel electrophoresis (PFGE) was performed on all isolates in order to compare their identity.
Results: Fourteen non-clonal strains were cultured from clinical samples including sputum (1), tracheal aspirate (2), wound (2), urine (5) and blood (4). All were non-susceptible to meropenem and imipenem, cefepime, cefotaxime, ceftazidime, and pip-tazo by the microtiter method. Seven, six and twelve were non-susceptible to amikacin, gentamicin and tobramycin, respectively. Only four were susceptible to ciprofloxacin.

Thirteen strains were susceptible to tigecycline (MIC $\leqslant 2\,\mu g/mL$). Strains were examined for the presence of carbapenemases using primers for blaKPC and blaOXA 23, 24, 25, 26, 27, 33, 40, 48, 49, 51 and 58, as well as metallo-β-lactamases. Amplicons were obtained with all strains with primers for blaKPC; sequencing revealed that 13 contained blaKPC-2 and one contained blaKPC-3. We also examined the ompk35, 36 and 37 porin genes in these strains. Sequencing data indicated that 9/14 strains contained a 1 bp insertion at NT245 in ompk35 resulting in a stop codon. The ompk35 sequence was absent in one strain. Most of the strains had from 3 to 10 aa long inserts into OmpK36 and 37 but these did not correlate with obvious functional disruption in the sequence of these porins.
Conclusion: Endemic outbreaks of multi-drug-resistant *K. pneumoniae* in NYC hospitals are becoming a concern and such resistant strains have spread to other areas of the Eastern USA. Presence of KPC-class carbapenemases in many of these strains account for high-level resistance to carbapenems and loss of functional outer membrane porins may contribute to resistance to these and other antimicrobials.

O497 Outbreak of carbapenem-resistant *Acinetobacter baumannii* isolates producing the carbapenem-hydrolysing oxacillinase OXA-97 (OXA-58-like) in a university hospital in Tunisia

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Objective: To characterise the clonal relationship and the β-lactamase content of carbapenem-resistant *Acinetobacter baumannii* isolates recovered in a University Hospital in Tunisia (Sousse) during a four-year period.
Methods: Forty-six non-repetitive carbapenem-resistant *Acinetobacter baumannii* isolates were recovered during the October 2001–October 2005 period from urine, pus, broncheal and blood samples. The presence of oxacillinase or metallo-β-lactamase genes was performed by PCR, cloning and sequencing. In particular, genes coding for the carbapenem-hydrolyzing oxacillinases (CHDL) OXA-23, OXA-40 and OXA-58 subgroups were searched. Genotyping was done by pulsed field gel electrophoresis (PFGE) after ApaI restriction.
Results: The *A. baumannii* isolates were mostly from patients hospitalised in intensive care units. MICs determination allowed to divide these isolates into two main phenotypical groups, both being resistant to most β-lactams including carbapenems. Twenty-two *A. baumannii* isolates possessed the blaOXA-97 gene encoding the novel carbapenem-hydrolysing oxacillinase OXA-97 differing from OXA-58 by a single amino acid substitution (Gly to Ala at position DBL35). OXA-97 exhibits the same hydrolysis profile as OXA-58, hydrolysing penicillins and carbapenems but sparing expanded-spectrum β-lactams. Genotyping revealed that the OXA-97-positive *A. baumannii* isolates belonged to a single clone, although six distinct genotypes were identified thus defining subclones. The blaOXA-97 gene was located on plasmids varying in size and they were all transferred to an *A. baumannii* reference strain by electroporation, whereas mating-out assays were unsuccessful. Only one isolate harboured the blaOXA-97 gene on its chromosome.
The other twenty-four carbapenem-resistant *A. baumannii* isolates did not produce any carbapenemase and they did not possess any CHDL gene. They were clonally related according to PFGE analysis.
Conclusion: This study constitutes the first description of dissemination of a CHDL enzyme in Africa and reports on the identification of the novel OXA-97 being closely related to OXA-58. As observed for OXA-58 in other countries, the gene coding for OXA-97 was mostly located on plasmids and identified in *A. baumannii* isolates being the source of an outbreak. Interestingly, this epidemiological survey showed that outbreaks of two different carbapenem-resistant *A. baumannii* strains had occurred in that hospital during the same period of time.

O498 Prevalence of AmpC among cephalosporin-resistant *Escherichia coli* and *Klebsiella* spp.

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Objective: To identify AmpC producing strains among *E. coli* and *Klebsiella* resistant to cephalosporins.
Methods: 152 *E. coli* and *Klebsiella* isolates identified as resistant to cephalosporins during routine examination for extended spectrum β-lactamase (ESBL) production were tested by combination disk method for inhibition by clavulanic acid to identify ESBL-producing strains. Further testing for susceptibility to cefoxitin and cefepime was done to identify putative AmpC producing strains. Strains resistant to cefoxitin were tested by the AmpC-disk test. This test discriminates between cefoxitin-resistance caused by cephamycinase activity in test-positive strains and other causes (e.g. loss of permeability or altered drug target) in test-negative strains.
Results: 68 of the 152 cephalosporin-resistant isolates exhibited resistance to cefoxitin and were classified as putative AmpC-producers. When further tested with the AmpC-disk test, 47/68 strains tested positive and were classified as AmpC producing; resistance to cefoxitin of the remaining 21 strains was not accountable for by β-lactamase production. 20/47 AmpC-disk test positive strains were inhibited by clavulanic acid and were classified as both AmpC and ESBL producing. These strains were significantly less sensitive to cefepime than the 26 strains not inhibited by clavulanic; the latter strains were classified as exclusively AmpC producing.
Conclusion: AmpC is prevalent in cephalosporin resistant *E. coli* and *Klebsiella* spp. In the present study 19% of the strains were classified as AmpC producing and of these strains 43% were also ESBL producers. Whether the latter group is truly AmpC producing or the positive AmpC disk test is the result of cephamycinase activity of the ESBL present is presently unknown. Molecular characterisation of the β-lactamases produced by the strains is in progress and may help resolve this question.

O499 Identification of CTX-M-15 extended-spectrum β-lactamase in a clinical isolate of *Shigella sonnei* in Ireland

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Objectives: To evaluate isolates of *Shigella* species from Ireland for Extended Spectrum β-lactamase (ESBL) production and associated antimicrobial resistance. ESBLs confer resistance to the oxyimino cephalosporins. In recent years the CTX-M β-lactamases, which preferentially hydrolyse cefotaxime, have been reported with increasing frequency worldwide. ESBL production in *Shigella* has previously been reported infrequently. Previous reports of ESBL production in *Shigella* spp. include a CTX-M-3 in *Shigella sonnei* in Turkey, CTX-M-2 in *Shigella flexneri* in Argentina, and CTX-M-14 in *Shigella sonnei* in Korea. This is the first report of CTX-M-15 in *Shigella* spp.
Methods: *Shigella* species received between January 2003 and October 2006 were tested for susceptibility to a panel of 14 antimicrobial agents including the following β-lactam agents: ampicillin, ceftazidime, cefotaxime, and cefpodoxime, in accordance with CLSI disk diffusion methods. ESBL production was confirmed by the CLSI combination disk method for ESBL production using cefpodoxime. Confirmed ESBL producers were screened for blaCTX-M by PCR using specific primers. Strains encoding blaCTX-M-9, M-15, M-25, M-2 were used as positive controls. Amplicons were sequenced using primers homologous to those used for PCR.
Results: Forty-six isolates of *Shigella* spp. (25 *S. flexneri*, 18 *S. sonnei*, 2 *S. dysenteriae*, 1 *S. boydii*) were received between January 2003 and October 2006. A single *Shigella sonnei* isolated from stool of an 8 yr old child was resistant to cefotaxime and cefpodoxime, and was confirmed as an ESBL producer in accordance with CLSI criteria. Co-resistance to ampicillin, streptomycin, sulphonamides, tetracycline, trimethoprim, and gentamicin was observed. No association with foreign travel was noted. PCR and sequencing revealed this isolate harboured a blaCTX-M-15.
Conclusion: This is the first report of CTX-M-15 in *Shigella* spp. CTX-M-15 has been reported extensively in clinical isolates of *Escherichia coli*. Its identification in *Shigella sonnei*, the leading cause of shigellosis in industrialised countries, is significant.