Emerging respiratory virus infections

S28 Avian influenza virus infections in humans

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Objectives: Recently a novel paramyxovirus isolated from children with respiratory tract disease was identified that was named human metapneumovirus (hMPV) and classified as a member of the metapneumovirus genus. We investigated the impact of hMPV infections and developed new tools and reagents for applied and fundamental research.
Methods: A large collection of hMPV isolates was generated, originating from different geographical locations from 1980 onwards. Sequence analyses were performed to study the genetic diversity of hMPV. Antigenic variation was measured with postinfection animal sera. A reverse genetics system was developed to produce recombinant hMPV. Improved diagnostic tests for hMPV were designed.
Results: Retrospective studies revealed that approximately 7% of the samples collected from individuals suffering from respiratory tract disease where positive for hMPV. HMPV was detected primarily in very young children and immunocompromised individuals and mostly in January and February. Clinical symptoms associated with hMPV infection in young children were found to be similar to those observed for RSV infections. In the 2000 and 2001 winter seasons in the Netherlands, only RSV was found to be associated with respiratory tract illnesses more frequently in an academic hospital setting. Sequence information on the fusion and attachment surface glycoproteins revealed that there are two main genetic lineages of hMPV (A and B) each of which, can be separated in two sublineages (A1, A2, B1 and B2). Virus neutralization experiments using animal sera indicated that the two main lineages represent different hMPV serotypes. We have sequenced the full-length genome of prototype strains representing each of these four genetic lineages. For both serotypes a prototype virus isolate was selected for which minigenome reporter systems were made and subsequently infectious recombinant hMPVs were rescued. We also developed and evaluated new diagnostic assays for detection of hMPV strains from all genetic lineages with equal specificity and sensitivity.
Conclusions: hMPV is an important viral pathogen. Young children, immunocompromised individuals and frail elderly are at risk for serious hMPV-associated disease. The genetic and antigenic heterogeneity of hMPV isolates should be taken into account for the development of diagnostic tests and evaluation of future vaccines. The applied and fundamental research on hMPV will benefit from the newly developed tools and reagents.

Sushi delights and leisure-related exposure

S39 Sushi and Sashimi risks

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'Sushi' and 'Sashimi' are the traditional Japanese dishes served with soya sauce dipping (3S Japanese food culture). Among three major types of 'Sushi' dishes, Nigiri, a piece of sliced raw fish fillet on vinegered rice ball, is the commonest dish served at Sushi bars. 'Sashimi' is an assorted dish of sliced raw fish fillets. Along with the popularization of Japanese dishes, 'Sushi' and 'Sashimi' are thought to be responsible in causing fish-borne parasitic zoonoses, especially anisakiasis, which is caused by infection with either herring worm, *Anisakis* sp. or cod worm, *Pseudoterranova decipiens*. The larval stages of them reside in the muscles and/or visceral organs of an array of marine fishes and squid. Human infection occurs by ingesting un-cooked/under-cooked marine products. However, apparent increase in anisakiasis cases in Japan and some other countries is related more to advances in diagnosis by endoscopy than to the epidemiological outbreak by commercialization of 'Sushi'. In addition, fish species used for 'Sushi' or 'Sashimi' are less contaminated with, or free from, *Anisakis* larvae. Of course, we should remind the high risk of infection with fish tapeworm (*Diphyllobothrium*) by ingesting salmon, which is commonly served as 'Sushi' and 'Sashimi'. The fish species having heavy burden of *Anisakis* larvae are cod, herring, mackerel and squids, which are cheap and mainly consumed at home. Concerning the risk of fish-borne parasitic zoonoses, we should pay more attention to the fresh- or brackish-water fishes. *Gnathostoma* larvae, which cause creeping disease, are common in the muscle of snakehead, cat fish, or tilapia. The disease is endemic in Asia, especially in Thailand and Japan. Recently an outbreak of this disease was reported from Mexico. Cultivation of tilapia in dam-lakes and its usage in traditional fish dishes, 'Cebiche' and 'Callos', are responsible for the outbreak. Consumption of freshwater crabs in Asia is at risk of infection with *Paragonimus*, a lung fluke. Many small fishes in fresh- or brackish-water carry metacercariae of minute intestinal flukes such as *Metagonimus, Heterophyes*, etc. In Asia, liver fluke larvae are found in freshwater fishes. Those flukes are usually harmless, but heavy infection occasionally causes serious GI symptoms. In conclusion, 'Sushi' and 'Sashimi' are rather at low risk of infection with zoonotic parasites. We need more attention on local ethnic dishes prepared by local freshwater fishes.

S41 Worms in south-east Asia: watch out what you eat and where you bath

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In the last decade south-east Asia has become an attractive place for tourists. Various activities are nowadays possible, reaching from beach holidays to eco-tourism exploring remote natural areas or visiting ethnic minorities. In south-east Asia a very frequent habit is the consumption of uncooked or partially-cooked foodstuff which favours the transmission of various parasites. As a result of the consumption of raw fish, opisthorchiasis is very common disease in the region. Although most liver flukes infections are asymptomatic, significant disease can be associated: obstructive jaundice, cholangitis, cholecysticis, cholelithiasis and specially cholangiocarcinoma have a high incidence in the region and contribute to the mortality. Less frequently, ingestion of raw fish can transmit gnathostoma or *Angiostrongylus* sp. These parasites are causes of eosinophilic meningitis and can also be found in frogs, snails and reptiles. Consumption of fresh rice field crabs enables the infection with *Paragonimus* sp.. The transmission takes place mostly is mountainous regions. Traditional medicine of ethnic groups can contribute to transmission as raw crab meat is given to children as fever-decreasing remedy. Erratic localization of eggs can cause neurological disease. Various dishes include inadequately cooked pork. A total of 120 epidemics of trichinellosis have been reported from 1962 to 2000 in Thailand, mostly in the North in Mong, Yao and Karen communities, involving 6700 patients and 97 death. In Laos, Thailand and north of Vietnam, recent studies have shown that cysticercosis is much more common than anticipated. Since the 1990s, fascioliasis is an emerging

disease in central provinces of Vietnam. Is it due to a raise of consumption of raw aquatic plants? Recent economic development enable the commercialization of cattle which favours the establishment of the transmission cycle. *Schistosomiasis mekongi*, is focally transmitted along the Mekong river in northern Cambodia and southern Laos. Although successful control programmes are implemented, transmission still occurs in certain areas; *S. mekongi* may lead to severe disease. A case of cerebral schistosomiasis has recently been reported in an exposed traveller. Prevention of food-borne parasitic infections should be an integral part in the counselling of travellers to south-east Asia and recommendations concerning the bathing in the Mekong river.

S42 Swimmer's itch: an emerging water-borne disease

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Swimmer's itch (cercarial dermatitis) is a common, noncommunicable infection that is widely neglected. The disease represents an allergic immune response which develops after repeated contacts of mammals (including man) with schistosome larval stages (cercariae). The most known causative agent of the disease are cercariae of the bird schistosome genus *Trichobilharzia* the life-cycle of which is connected with fresh-water bodies all over the world.
In the past, it has been assumed that the parasites die soon after the penetration into the noncompatible host skin. However, recent observations on bird schistosomes showed that the immature flukes are able to escape from the skin and migrate further to internal organs where they can survive for several days and even weeks in mammals under certain circumstances. And depending on the species, particular schistosomes can cause severe tissue injuries either in the lungs or in the central nervous system (CNS) of these type of hosts. Our studies on *Trichobilharzia* infections showed that the involvement of internal organs during bird schistosome infections in mammals is dependent on the host-immune status. During primary *T. regenti* infection of immunocompetent mice, the parasites evoked acute inflammatory reaction in the skin and the CNS involving focal oedema and cellular infiltration of the tissues. Challenge infections resulted in the development of extensive inflammatory foci in the host skin which precluded the subsequent migration of the schistosomula to the CNS. However, during primary and challenge infections of immunodeficient hosts (SCID mice), no significant immune response against the parasites in any of examined host organs was detected; however, in contrast to immunocompetent mice, the infections were frequently manifested by severe neurological symptoms. Moreover, the studies on mice with various immune status showed that the deficiency in T- and B-cell immune response mice can lead to longer survival of the parasites in SCID mice. Our results showed that the infection of mammals with bird schistosomes may cause more serious problems than just cercarial dermatitis alone in certain cases. The cosmopolitan occurrence of *T. cercariae* and increasing exposures of humans to these parasites show the necessity to intensify the studies on these parasites.

Intervention to improve antibiotic prescription

O47 Improving the management of inpatients with community-acquired pneumonia: controlled before and after study

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Objectives: Community-acquired pneumonia is a common cause of acute medical admission. We have previously identified time to antibiotic administration and antibiotic appropriateness as potentially important quality improvement targets (*Clin Infect Dis* 2002; 34: 218–223). The aim of the project was to implement a quality improvement intervention and to evaluate its impact on processes and outcomes of care.
Methods: Preimplementation, qualitative and quantitative methods identified barriers to the efficient delivery of appropriate antibiotics. These data were used to design a targeted intervention (wall-based pathways, promotional posters, implementation packs, educational sessions and audit and feedback). Process of care and outcomes were compared at one study and one control hospital over two winters (2001/02 and 2002/03), before and after the intervention. The primary outcome was the proportion of patients receiving appropriate antibiotics (British Thoracic Society definition) within 4 h of admission. Cost effectiveness analysis was performed from the hospital's perspective.
Results: The proportion of patients receiving appropriate antibiotics within 4 h increased by 70% at the study hospital: from 33% (60/181) to 56% (118/209) vs. a 10% increase at the control hospital: from 32% (19/60) to 36% (19/53). Unadjusted absolute change (study − control) = 20%, $P < 0.01$; adjusted absolute change = 17%, $P = 0.035$. This difference was mostly because of an improvement in 'door to antibiotic time', the proportion of patients receiving appropriate antibiotics by BTS criteria improved similarly at both hospitals. The improvement in door to needle time was not associated with any significant difference in length of hospital stay or mortality between the study and control hospitals ($P = 0.4$ adjusted for severity). The cost per additional patient receiving appropriate antibiotics within 4 h was £1776 for a project with full evaluation but could be reduced to £132 by implementing the intervention without full evaluation. Costs would also be substantially reduced if outcomes could be evaluated from electronic medical records.
Conclusions: The intervention significantly improved the quality of initial care, but at notable cost. Costs would fall considerably if routinely collected electronic data were available. This is the second intervention study that has failed to show an association between decreased door to needle time and improved outcome of CAP.

O48 The path of least resistance: the value of educating patients and professionals about antimicrobial resistance

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Objectives: To evaluate the impact of a digital library on changing patient and professional knowledge and attitudes to antimicrobial prescribing.
Methods: Research has shown that patient expectation and the perceived expectation by the doctor influences general practice prescribing of antibiotics. Both the SMAC report 'The Path of Least Resistance in 1997', and the House of Lords report in 1998 highlighted the importance of educating the public about antimicrobial resistance as part of the strategy to contain it. So how can we provide timely access to quality-controlled information for both professionals and patients and know that it is making a difference to their knowledge and attitudes? The National Electronic Library for Health (NeLH) is a freely accessible Internet gateway to the best available evidence. At City we are

developing the infection specialist library (NeLI). The key value of the library lies in the quality appraisal of documents by expert reviewers. One aim of NeLH is to provide health care professionals and the public with knowledge and know-how to support health care related decisions. But does NeLI have any influence on user knowledge and attitudes about antimicrobial resistance? We used pre and postuse questionnaires to evaluate the impact of NeLI on users.
Results: The study was conducted in several different locations. At the Science Museum, London 177 visitors completed both questionnaires. There were statistically significant increases in knowledge and reductions in expectation of receiving antibiotics for acute otitis media (AOM). The most significant change was from 10 to 46% of users correctly saying that people cannot become resistant to antibiotics. The percentage of users agreeing that doctors should usually prescribe antibiotics for a child with AOM fell from 51 to 33% and for those agreeing that they would expect an antibiotic for themselves or their child with AOM fell from 59 to 30%. Similar results were obtained with a smaller group at Nottingham City Hospital. The study has recently been repeated at Oxford University Medical School and results will be available for the conference presentation.
Conclusion: To summarise, there were significant improvements in user knowledge about antibiotics, after using the site users had a reduced expectation of receiving antibiotics for AOM. Internet resources can be effective tools in improving health outcomes.

O49 Attitudes to antibiotic prescribing, resistance and laboratory investigations amongst practitioners and patients in the Grampian region of Scotland

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Objectives: Patient pressure and difficulties in accessing laboratory facilities are often put forward as reasons for inappropriate antibiotic prescribing in the community. Community general practitioners (GPs) and patients were questioned to establish their perception of appropriate prescribing, the existence of resistance problems and the use of laboratory investigations to facilitate prescribing.
Methods: The Grampian region of Scotland operates an accelerated bacteriology laboratory examination (ABLE) service, ensuring overnight processing of samples in order to allow informed antibiotic prescribing. During an audit of use of the service and of antibiotic prescribing, we distributed questionnaires to both GPs and patients. Questions related to attitudes and knowledge of antibiotic prescribing and resistance and feedback on the ABLE service. Results were entered in an Excel spreadsheet and analysed by SPSS.
Results: A total of 49 GPs responded (response rate 90%). 89% thought that reducing prescribing reduces resistance but only 38% considered resistance a problem in their own practice. 92% used the ABLE service, but only 8% daily and 47% weekly. 67% said they combined the service with a delayed prescription. 53% thought it decreased antibiotic prescription rates. 701 patients were surveyed. 23% expected antibiotic for a sore throat, 36% for a chesty cough, 35% for earache and 17% for sinusitis. Notably, expectations were markedly lower for their children. 88% thought the ABLE service was a good idea. 50% thought resistance a big problem and 74% wanted more information on antibiotic resistance in the form of a leaflet.
Conclusions: Acceptance of the ABLE service and awareness of antibiotic resistance was high. Patient expectations of antibiotics were low compared with the literature and provide a good grounding for further improvements in antibiotic prescribing based on decreased patient pressure and increased use of ABLE. Both GPs and patients seem well placed to maintain recent improvements in antibiotic prescribing in the Grampian region.

O50 Antimicrobial usage and resistance trends among Gram-negative nosocomial bacteria: a 4-year follow-up study in a Belgian hospital

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Objective: An increase of extended-spectrum beta-lactamase (ESBL) producing *Enterobacter aerogenes* (E.a.) and multi-resistant *Pseudomonas aeruginosa* (PSA) was documented in our hospital during the 1998–2000 period. In 2001, an antibiotic policy aimed to control the usage of broad-spectrum antibiotics was instituted. We aimed to compare the antimicrobial usage and the prevalence of resistance among nosocomial Gram-negative bacilli (GNB) isolates between 2000 and 2003 in order to evaluate the relationship between antibiotic use and resistance prevalence in nosocomial infections.
Methods: Resistance data were collected from all GNB strains isolated from nosocomial infections. Antibiotic consumption data were monitored with the ABCCalc software according to the ATC/WHO 2003 methodology and data were expressed in prescribed daily doses (PDDs)/100 bed instead of defined daily doses (DDDs).
Results: The global antibiotic consumption decreased by 14% (50.9 PDDs in 2003 vs. 58.8 in 2000), the largest decrease being noted for the fluoroquinolones (FQs) (7.0–4.5 PDDs; -36%), especially the parenteral formulations (2.9–0.8 PDDs; -72%). Decreased usage was also recorded for cefuroxime (7.6–5.2 PDDs), ceftazidime (1.9–1.0 PDDs) and meropenem (2.2–0.8 PDDs) while the use of cefepime increased from 2.2 to 2.9 PDDs. No changes were observed for ureidopenicillins, aminoglycosides and glycopeptides. A decline in resistance was observed through the study period for beta-lactams (piperacillin/tazobactam −15%, cefuroxime −14%, ceftazidime −9%), ciprofloxacin (19%) and amikacin (8%) among Enterobacteriaceae isolates. These lower resistance rates were essentially linked to a marked reduction in the proportion ESBL-producing E.a. over time (14.3–7.6%; −49%). In PSA, the proportion of multi-resistant strains fluctuated but did not vary significantly, the only trend observed being a lowering in resistance rates to gentamicin (17%) and tobramycin (8%) while no changes were recorded for other anti-pseudomonal agents.
Conclusions: The enforcement of a controlled antibiotic policy led to a significant decrease in the consumption of broad-spectrum antibiotics. A direct relationship could be observed between the decreased usage of FQs over time and a marked decline in the number of ESBL-producing E.a. isolates. Our study illustrates that controlled usage of broad spectrum antibiotics may have a positive impact on the epidemiology of nosocomial bacteria.

O51 Interventions to improve vancomycin prescribing – a useful exercise or a waste of time?

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Therapeutic drug monitoring has become a standard of care in patients on vancomycin, despite controversary regarding its role in preventing toxicity and optimizing outcome. Informal observations by the clinical microbiology team in our institution, suggested that patients were not receiving all doses of vancomycin prescribed. As a consequence, we conducted a prospective audit of vancomycin usage and assessed the impact of therapeutic drug monitoring on administration of vancomycin in our institution. The initial month-long audit revealed that the majority of patients (51/54) were prescribed vancomycin appropriately. However patients received only 725 of 1007 prescribed doses (72%). Reasons for failure to administer 282 doses included no

i.m. access (3%) and toxic levels (39%). However in the remaining 58% the antibiotic was withheld inappropriately either awaiting level results or for no apparent reason. This study highlighted the confusion among health care professionals about administration and laboratory monitoring of vancomycin. As a consequence, our department undertook several measures in order to improve vancomycin prescribing and laboratory monitoring. These measures included feedback of audit findings to medical, nursing, pharmacy and management staff at hospital-wide educational meetings, changes in laboratory computer and phlebotomy practices and changes in microbiology department policy with regard to level ordering, frequency and interpretation of levels. One of the changes that we undertook was to abolish random, peak and trough testing options in favour of a single predose level. This simplified level ordering, which was a major source of confusion among medical staff. A second audit of practice is currently underway. This presentation will discuss the success or otherwise of our interventations and highlight problems that we identified whilst attempting to improve vancomycin prescribing and level ordering in our institution.

O52 A computerised decision support system (TREAT) to reduce inappropriate antibiotic therapy of bacterial infections

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Background: Inappropriate empirical therapy of bacterial infections is associated with an increase in fatality rate. TREAT is a computerised decision support system (DSS) for antibiotic treatment of bacterial infections in in-patients. It looks at risk factors for infections, local distribution and susceptibilities of bacteria, and then balances the benefit of antibiotic treatment against its costs to advice on a drug.
Objectives: To improve the percentage of appropriate antibiotic treatments by using the TREAT system in clinical practice.
Methods: A multi-centre observational clinical trial has been performed at three University hospitals. All consecutive adult patients for whom antibiotic treatment was started have been included over a 7-month study period. The following variables were collected before starting antibiotic therapy, at the availability of microbiological result, and at final diagnosis: comorbidies, risk factors for infections and pathogens, clinical and microbiological data, physicians' chosen therapy and TREAT advice. Primary outcome was the percentage of appropriate empirical antibiotic treatment, defined as antibiotic treatment matching the susceptibility of the isolated pathogen. Test-based confidence intervals (CI) at 95% were used to determinate the significance of the relative risk (RR).
Results: During the observational trial, 1203 patients were enrolled. Infections were acquired in the community in 70% of cases. The most frequent outcome diagnosis were: pneumonia (24%) and septicaemia (11%). Significant clinical isolates were available in 352 patients (29%) with the most frequent being: *E. coli* (36%), *S. aureus* (17%), and *P. aeruginosa* (16%). Appropriate antibiotic treatment was given by TREAT to 63% of patients, compared with 56% of treatments prescribed by physicians (OR 0.33, 95%CI 0.07–0.53, *P* 0.01). The use of the programme led to significant decrease in inappropriate treatment from 38 to 28%. The overall cost of treatment prescribed by TREAT was 58% (95% CI, 56–59%) of the treatment cost prescribed by physicians. The direct antibiotic cost per patient for 1 day was 17.5 Euro for TREAT vs. 31 Euro for physicians.
Conclusions: TREAT achieved a significant increase in appropriate empirical antibiotic treatment of patients with bacterial infections. These results seem to suggest that computerised DSS using local data can streamline appropriateness of antibiotic therapy and reduce hospital costs.

O53 Impact of an intervention programme to reduce inappropriate antibiotic prescriptions in south-eastern France

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Background: France has very high pneumococcal resistance and antibiotic consumption rates. Nasopharyngeal (NP) nonpenicillin-susceptible pneumococcal (NPSP) carriage rates in children's day-care centres in the Alpes Maritimes (AM) and Nord (N) areas reached 60 and 72%, respectively, during Winter 1999. A local intervention programme was implemented in AM in 2000 to reduce antibiotic prescriptions in paediatric upper respiratory tract infections (URTI).
Methods: Resistance data and guidelines were provided to general practitioners and paediatricians in AM during peer-conducted academic detailing visits. Parents received an information leaflet on bacterial resistance. A new cross-sectional survey of NPSP carriage was conducted in AM and N during Winter 2002. NP samples were collected and parents were questioned about AB treatment during the past 3 months.
Results: Between 1999 and 2002, among SP carriers, the proportion of NPSP remained stable in AM: + 0.02% (ns). In N it increased by 19% ($P = 0.01$). Between 1999 and 2002, the proportion of treated children in AM decreased by 18% ($P = 0.03$) and remained stable in Nord: +2.2%, (ns). Sentinel surveillance of URTI did not show differential epidemic contexts between the two areas.
Conclusions: Academic detailing visits promoting prudent antibiotic use appear to have been effective in reducing prescription rates for children attending day care.

O54 Implementation of multidisciplinary antibiotic management teams in Belgian hospitals: pilot phase evaluation, 2002–03

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Objectives: To analyse the interventions undertaken by Belgian hospitals during a pilot phase of supporting local antibiotic managers (AM) and multidisciplinary antibiotic management teams (AMT).
Methods: In 2002–03, the Belgian government subsidised the part-time activities of AMs in 35 hospitals selected based on the presence of an operational AMT. The activities described in the first 8-month progress reports were analysed according to national guidelines for AMTs.
Material: The pilot hospitals had a median capacity of 654 (range, 154–1597) beds; 18 hospitals comprised several sites. Their regional distribution was representative of population size, with 18 hospitals located in Flanders, 11 in Wallonia and six in Brussels; 17 were general hospitals, eight teaching hospitals and 10 general hospitals with teaching beds.
Results: AMs were trained as internists (28), microbiologists (13) and hospital pharmacists (13). In 18 hospitals the AM also served as infection-control physician. On average, AMTs included 10 members who met every 6 weeks. The pilot financing scheme allowed the implementation of 175 antibiotic management interventions, with a mean of five interventions per hospital. More interventions were launched in large size and teaching hospitals. All hospitals irrespective of size or affiliation had undertaken a wide range of measures: review of formulary (29), implementation of new clinical guidelines (24), restricted access to select antibiotics (25), improvement of susceptibility-testing methods (12), development of antibiotic consumption database (35) and analysis of antibacterial susceptibility data (31). Updated guidelines addressed mainly urinary tract infection (14), pneumonia (14) and meningitis (10) and indications for use of specific drugs (14). Use of glycopeptides, carbapenems, fluoro-quinolones, cefepime and ceftazidime was restricted in respectively 16, 12, 12, 12 and 10 hospitals.

Conclusion: All hospitals that received financial incentive under the AMT pilot scheme have developed multiple antibiotic policy interventions independently of the hospital size and teaching status. Extension to all Belgian hospitals appears warranted. Further analysis is planned to monitor the impact of this scheme on the use of antibiotics and trends of antibiotic resistance.

O55 Antimicrobial availability for oncology patients with advanced malignancy in Latin America: WHO/PAHO Medical Oncology Survey Report 2000–2002

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Objective: Evaluate availability of major classes of antimicrobials to physicians providing health care to patients with advanced cancer in Latin America.
Methods: A cross-sectional descriptive analytic survey was conducted from March 2000 to November 2002. The self-administered anonymous survey was approved by institutional review board of The University of Texas M. D. Anderson Cancer Center. The six major groups of antimicrobials surveyed included: aminoglycosides, antifungals, beta-lactams, quinolones, trimethoprim-sulphamethoxazole and vancomycin.
Results: Responses were obtained from 936 physicians including medical and surgical oncologists, anaesthesiologists and palliative care/pain management specialist from 26 countries. Most respondents (>90%) practiced in urban setting. Most responses were received from Brazil ($n = 345$; 37%), Mexico ($n = 129$; 14%), Argentina ($n = 108$; 12%), and Cuba ($n = 59$; 6%). The mean response from Argentina, Brazil, and Mexico generally rated the availability of all antimicrobial classes as 'Good'. Respondents from Mexico, however, despite belonging to the same GNP (gross national product group III, $ < 7000) as Brazil, reported availability of vancomycin significantly lower than respondents from Brazil ($P < 0.000$). Physicians from Cuba (GNP group IV, $ < 5000), in addition, rated all classes of antimicrobials as significantly lower compared with responses from the other three countries, with the exception of trimethoprim-sulphamethoxazole ($P < 0.0001$).
Conclusions: In Cuba, reduced availability of all major groups of antimicrobials for the treatment of patients with advanced cancer is a concern. This study provides important information in reassessing and re-establishing future guidelines for regional and global healthcare support stratagies.

O56 Household survey of residual antibiotics and beliefs about antibiotics

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Objectives: To measure the extent to which entirely or partially unused prescribed antibiotics are retained in UK households and to elicit the reasons for such retention. To assess the public's awareness of antibiotic resistance and the content of publicity campaigns.
Methods: We added additional questions to the Omnibus survey, conducted monthly throughout Great Britain for 8 months each year by the Office of National Statistics. Our surveys were carried out in February/March and June/July 2003. Adults aged 16 years or over living in private households were approached by letter for face-to-face interview based on a stratified probability sample of the population. Interviewers had no control over who was interviewed - they had to interview at the preselected address and select one adult for interview according to a preset method. Unlike quota samples this method avoids the bias introduced when interviewers select only people who are easy to persuade to take part in the survey. The area in which each house was located was ranked by the ACORN geo-demographic classification.
Results: A total of 7319 households were identified and 7120 (97%) took part in the survey. Of these 1316 (18%) had 1659 antibiotics in the house. Of the 7120 households, 365 (5%) held antibiotics that were partially unused from a previous prescription, 219 (3%) held antibiotics that were authorised by a doctor to be kept for a future infection, 727 (10%) held antibiotics that were currently in use and five were unsure. There was no relationship between geo-demographic area and residual antibiotics: 5%, 95% CI 4–6% least-deprived; 5%, CI 4–6% most-deprived. However, people in the least-deprived areas were less likely to believe that antibiotics work against most coughs or colds (27%, CI 25–28% vs. 35%, CI 34–37%), more likely to believe that bacteria on their skin or in their gut are good for their health (64%, CI 62–66% vs. 53%, 51–55%) and more likely to believe that antibiotic resistance is a threat to them or their family (84%, CI 83–85% vs. 80%, CI 78–81%).
Conclusions: Few households retain prescribed antibiotics for future use. Although a majority of people believe that antibiotic resistance is a threat to them or their family a substantial minority still believe that antibiotics work against most colds or coughs and there is a significant relationship between geo-demographic classification and educational need.

Novel molecular methods for bacteriological diagnosis

O57 A FISH assay with peptide nucleic acid probes for direct identification of blood cultures: evaluation in the routine setting

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Objective: Rapid identification of blood isolates is a key to the management of bacteraemia. The Gram stain is crucial, but a definite diagnosis must await subculture and supplementary tests. Identification within 3.5 h has recently become feasible by use of peptide nucleic acid (PNA) probes for fluorescence-*in situ*-hybridization (FISH). We have evaluated this technique for identification of *S. aureus* (Sau), *E. coli* (Eco), *P. aeruginosa* (Pae), and *C. albicans* (Cal).
Methods: The evaluation was conducted May–November 2003. BacT/Alert (bioMérieux) was used for blood cultures (BCs) and a set with two aerobic and one anaerobic bottle was recommended for adults. PNA probes were provided by AdvanDx (Woburn, MA, USA), and PNA-FISH assays were carried out with flame-fixed slides. The Gram stain report determined which probes were used (the Sau probe for Gram-positive cocci in clusters, Eco and Pae probes for Gram-negative rods, and the Cal probe for yeasts). The observer was blinded to conventional identification. Positive and negative predictive values (PPN and PNV, respectively) were calculated for monomicrobial cultures and numbers of patients with homologous and heterologous isolates are given in [ ].
Results: Among app. 9000 BC sets 1241 produced growth. Based on Gram stain results 789 BCs from 534 patients entered the evaluation. Diagnoses provided (or rejected) by PNA-FISH were in agreement with conventional diagnoses for the following groups of bacteraemic patients: *S. aureus* (60) vs. coagulase-negative staphylococci (127), *E. coli* (197) vs. other Gram-negative rods (145), and *C. albicans* (9) vs. non-*C. albicans* (10). Thus, PPV was 100% and NPV 100% for the Sau, Eco, and Cal probes. PNA-FISH with

the Pae probe was positive in 18 of 19 patients with *P. aeruginosa*; weak to moderate staining was seen in four patients with other aerobic bacteria, and 316 patients with a broad range of Gram-negative rods were negative. The PPV for the Pae probe was 82% and NPV 99.7%.
Conclusion: PNA-FISH is speedy and the diagnosis of *P. aeruginosa* is imperative in order to provide coverage for this pathogen. PNA-FISH was found to be highly reliable in the routine setting for diagnosis of four important blood-borne pathogens. For all four pathogens the NPV was > 99% and the slightly lower PPV for *P. aeruginosa* should not limit the clinical utility of PNA-FISH.

O58 Polymerase chain reaction for the diagnosis of osteoarticular infections due to *Kingella* or other fastidious organisms in paediatric patients

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Introduction: Despite the improvement of bacteriological methods, synovial fluid and bone biopsy specimens remain negative in a substantial fraction of patients with clinical osteoarticular infections. This work evaluated the potential usefulness of a molecular approach in the diagnosis of osteoarticular infections due to fastidious organisms in paediatric patients.
Patients and Methods: From January 2001 to March 2003, osteoarticular specimens from 182 paediatric patients (age range: 1 month—17 years, median: 3 years and 7 months) were inoculated onto conventional media in our microbiological laboratory. Moreover, all the liquid specimens were inoculated into Bact/Alert FAN blood culture bottles. On clinical arguments of infection, 77 culture-negative specimens were tested by an universal 16S rDNA PCR method followed by direct sequencing of DNA to identify microbial pathogens.
Results: A positive culture was obtained for 49 specimens (26.9%) including 22 *Staphylococcus aureus* (44.8%), eight beta haemolytic *Streptococcus* (16.3%), eight *Kingella kingae* (16.3%), four *Streptococcus pneumoniae* (8.2%) and seven miscellaneous organisms (16.3%). A positive PCR result was obtained for 15 of the 77 culture-negative specimens tested (19.5%). For 12 PCR-positive specimens, the sequencing allowed the identification of organisms of the Neisseriaceae family (including the different genus *Kingella* spp., *Eikenella* spp., *Simonsiella* spp. and *Alyssiella* spp.) of which two *Kingella kingae* and one *Eikenella corrodens* strains were fully identified at the species level. For three PCR-positive specimens, the sequencing was not possible due to unsufficient amount of DNA. Both patients either with culture-positive *Kingella* specimens ($n = 8$) or PCR-positive specimens ($n = 12$) had similar pathologies (15 arthritis, three osteitis and two spondylodiscitis) and corresponded to a young patient population (age range: 8 months—4 years, median 1.5 year) encompassing 13 boys and seven girls.
Discussion: While fastidious bacilli such as *Kingella kingae* were considered as an exceptional cause of osteoarticular infections, recent improvement of culture methods have demonstrated that these organisms were more common than previously recognised. In this study, compared with the culture, PCR increased the recovery rate of fastidious *Bacillus* from 16.3 to 46.9%.
Conclusion: PCR is a powerful tool since it contributed to the diagnosis and managment in 15 of 77 culture-negative osteoarticular infections.

O59 Improved diagnosis of listeriosis by real-time TaqMan-based PCR

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Objectives: The food-borne pathogen *Listeria monocytogenes* is capable of causing severe and fatal infections in certain high-risk patient groups including meningitis, encephalitis, septicaemia and gastrointestinal symptoms. The organism is very widely distributed in nature with consumption of contaminated foods being the major route of transmission. A definitive diagnosis of listeriosis depends upon culturing the organism from the blood or cerebral spinal fluid (CSF). However, low numbers of the bacterium may be present in both samples and problems with culturing this bacterium after commencement of antimicrobial therapy are well recognised. The aim of this study was to develop a sensitive, real time PCR assay to rapidly confim the identify of isolates, and to detect *L. monocytogenes* specific nucleic acid in patient's CSF or serum.
Methods: Real time TaqMan-based PCR assay (both nested and un-nested) to specifically amplify a fragment of the *L. monocytogenes hly* gene was established.
Results: The assay proved very useful for the rapid confirmation of *L. monocytogenes* colonies growing *in vitro* and was specific for this bacterium. Since April 2002 more than a 1000 isolates of *L. monocytogenes* from food and clinical samples have been identified as *L. monocytogenes* using this assay. Following its successful introduction the assay was then assessed for detecting *L. monocytogenes* in clinical samples from patients with clinical symptoms of listeriosis. The sensitivity of the assay was further improved by performing a 25 cycle PCR assay prior to the real time assay. Although this step increases the time to get results it significantly improves sensitivity. Of 26 DNA samples extracted from either serum or CSF only three were positive for *L. monocytogenes* by the single round TaqMan PCR assay. Four DNA samples gave equivocal results and 19 were negative. Whereas, by the nested TaqMan-based PCR assay, 15 samples were positive for *L. monocytogenes*, zero gave equivocal results and 11 were negative.
Conclusions: These results show that the assays are useful for rapid confirmation of cultures growing *in vitro*, and may also be applicable for detection in enrichment broths. The nested assay will provide an additional tool for the establishing of a noncultural diagnosis of listeriosis, especially in patients with suspected neurological infections.

O60 Evaluation of diagnostic methods for *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* using serology, PCR and the BD ProbeTec ET System

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Objective: To evaluate diagnostic methods including the BD ProbeTec ET System (ProbeTec), conventional PCR and serology for *M. pneumoniae* and *C. pneumoniae* in outpatients with persistent cough.
Methods: A total of 193 patients from 12 Swedish primary care centres were included. The mean duration of cough was 24 days. Mean age was 45 years (range 8–90). Acute and convalescent serum samples were obtained from 170 of the 193 (88%) patients. IgG, IgA and IgM antibodies towards *C. pneumoniae* were detected by EIA (ThermoLabsystems). A positive result was defined as seroconversion for IgA or IgG or IgM. Serology for *M. pneumoniae* was performed with the particle agglutination test kit of Anti-*M. pneumoniae* antibody (Fujirebio). A positive result was defined as a fourfold rise in titre. Sampling from the posterior pharyngeal wall was performed with two separate swabs, one for ProbeTec and one for the Rapidcycler PCR (Idaho Technology). Primers from the 16S rRNA gene were used for PCR amplification of *C. pneumoniae* DNA according to Gaydos *et al.* (1992). For PCR detection of *M. pneumoniae* DNA, primers for the P1 adhesin gene were used. The BD ProbeTec ET System *M. pneumoniae* (MP Assay) and Chlamydiaceae family (CF assay) are based on real-time homogenous strand displacement amplification and detection technology. The sensitivity and specificity of the CF and MP assays were calculated using a combined reference of both PCR and serology. True positives were defined as positive by both reference methods. True negatives were defined as negative by both methods.
Results: Of the 193 patients, three were not tested with the CF or MP assays. Results of the remaining 190 patients are listed in the table below by PCR, serology and ProbeTec Assay.

Conclusion: There was a very good correlation between the molecular and serologic tests. The CF Assay and MP Assay are suitable for the rapid diagnosis of atypical pneumonia.

	PCR and Serology positive	PCR positive only	Serology positive only	PCR and serology negative	Total
CF Assay positive	8	3*	1*	0	12
CF Assay negative	1	3*	1*	173	178
CF Assay sensitivity			88.8%(8/9)		
CF Assay specificity			100%(173/173)		
MP Assay positive	9	3*	0	1	13
MP Assay negative	0	0	2*	175	177
MP Assay sensitivity			100%(9/9)		
MP Assay specificity			99.4%(175/176)		

*These specimens were not included in sensitivity/specificity calculations since they were only positive by either serology of PCR, but not both.

O61 Application of different nucleic acid amplification techniques for the detection of *M. pneumoniae*, *C. pneumoniae* and *Legionella* spp. in respiratory specimens

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Introduction: The advantage of nucleic acid amplification techniques (NAAT) is their extreme sensitivity and specificity when compared with traditional techniques. Multiplex formats might solve the practical shortcoming of detecting only the infectious agent that is searched for. Real-time assays enable a one-tube assay that is suitable for high-throughput applications, reducing the assay time and limiting potential contamination between samples. A comparison between different NAAT is needed.
Objectives: To compare the sensitivity and specificity of real-time (RT) mono- and multiplex nucleic acid sequence-based amplification (NASBA), and mono-PCR for the detection of *M. pneumoniae*, *C. pneumoniae* and *Legionella* spp. in respiratory specimens from hospitalised and outpatients with community-acquired pneumonia (CAP).
Methods: A total of 244 respiratory specimens were collected from 142 patients with CAP (116 throat swabs, 112 sputa and 16 other respiratory specimens). NASBA was done by using the NucliSens Basic Kit (bioMérieux), PCR was done as described earlier. PCR detects *L. pneumophila* whereas NASBA was designed to detect *Legionella* spp. All samples with discordant results were reanalysed. Definition of the expanded gold standard used to calculate the sensitivities of the tests applied: true positive if positive by at least two NAAT.
Results: The sensitivities of the different techniques, compared with an expanded gold standard, were 77.8, 100, and 100% for detection of *M. pneumoniae*; and 50, 100, and 50 for detection of *L. pneumophila* by PCR, RT mono-NASBA, and RT multiplex NASBA respectively. If positive by any method, the sensitivities were 63.2, 92.1, and 71.1 for detection of *M. pneumoniae*; 57.1, 71.4 and 28.6 for detection of *L. pneumophila* by PCR, RT mono-NASBA, and RT multiplex NASBA, respectively. Two samples were found to be *C. pneumoniae* positive only by RT mono NASBA which is an insufficient number to calculate the sensitivity of the tests applied.
Conclusions: Mono RT NASBA is more sensitive than mono PCR. When the former is compared with RT multiplex NASBA, the mono NASBA is the most sensitive test. This confirms results presented earlier by using spiked clinical specimens. The RT multiplex NASBA could become a fast, and user-friendly diagnostic tool for the detection of these organisms in respiratory specimens although it seems to be less sensitive. Further comparison on larger numbers of clinical specimens is necessary.

O62 Consensus real-time PCR assay for *Bordetella pertussis* including an internal process control provides, specific diagnosis, rapid turnaround time, high diagnostic yield and enhanced surveillance

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Objectives: (i) To transfer existing block-based PCR detection of *Bordetella pertussis* to a real-time platform; (ii) to offer this as a diagnostic test for *B. pertussis* by the Respiratory and Systemic Infection Laboratory, Health Protection Agency, London, as part of a range of reference and enhanced surveillance tests useful in the investigation of individual cases and outbreaks of pertussis infection.
Methods: A consensus PCR approach was used; LightCycler (Roche) assays for two gene targets were developed using hybridisation probes for confirmation of products: (i) pertussis toxin promoter (*B. pertussis*-specific) together with an internal process control (IPC) and (ii) insertion element IS481 (which occurs in *B. pertussis* and *B. holmesii*). This service is offered free of charge for testing nasopharyngeal aspirates from children, age 6 months or below, with respiratory illness compatible with pertussis, admitted to paediatric intensive care units or paediatric wards. Real-time assays were introduced in October 2001, with an official launch date in 2002. The QiaAmp DNA Mini Kit (Qiagen) was used for extraction and samples were tested at three dilutions, neat, 1:10 and 1:100 (in nuclease-free water). Samples were considered to be positive for *B. pertussis* if both targets were detected.
Results: From October 2001 to October 2003, 346 patient samples were received for *B. pertussis* detection. Of these 269 (78%) had no genomic evidence of *B. pertussis* infection (i.e., negative for both targets), 51 (15%) had evidence of recent *B. pertussis* infection (i.e., positive for both targets), 49 (14%) samples showed some inhibition resulting in a reduced sensitivity of the assays, five (1%) were total inhibitory, 16 (5%) were scored as equivocal (i.e. positive for only one target).
Conclusion: Successful transfer of assays to a real-time platform was achieved resulting in a more rapid turnaround time. Typically, a same-day service for samples received before 10 AM was achieved. Inclusion of an IPC prevented the reporting of false-negative results. Although the IS481 assay is not *B. pertussis*-specific, it is more sensitive that the toxin-promoter assay. Thus, a consensus PCR approach provided specificity and increased confidence in the results. Together with serological results and culture isolation, real-time PCR is a valuable tool for the enhanced epidemiological surveillance and diagnosis of pertussis and allows a rapid turnaround time.

O63 MRSA screening by RT-PCR directly from the swab within the same day

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Objectives: More than 50% of all patients with MRSA of the Regensburg University Hospital are already positive on admission. Isolation of patients results in higher workload, costs and reduced patient care. The goal of this study was to accelerate the

results of MRSA screening by real time PCR in patients with high or low risk for MRSA carriage.
Methods: MRSA screening by RT-PCR directly from the swab was compared with culture and Pbp2a antigen detection from overnight culture. All patients admitted to any ICU of the Regensburg University hospital and at low or high risk to be colonised with MRSA were included into the study. One swab for culture and PCR each from both nares were taken. MRSA was cultured on sheep blood agar and mannitol salt agar and identified by Slidex Staph and Slidex MRSA kit (Biomerieux, Nürtingen). DNA was extracted automatically by the MagNA Pure instrument with the MagNA Pure Microbiology Kit, Mgrade, detection and differentiation of *S. aureus* and CNS was performed with the LightCycler (LC) *Staphylococcus* Kit Mgrade, mecA by the LC MRSA Detection Kit (Roche, Mannheim).
Results: A total of 38 of 456 (8.3%) of all patients enrolled were at high risk for MRSA-carriage and isolated. More than 50% of all patients were on antibiotics. Main reasons for MRSA screening were multimorbidity (60%) and prior hospitalisation (18%). A total of 450 of 456 patients could be evaluated for the comparison of tests. In culture and PCR MRSA was positive in 31 of 450 (6.8%) and 29 of 450 patients respectively (sensitivity 94%). When using culture as gold standard sensitivity was 83% (70 of 490). Only two of 450 samples were false negative in PCR (0.4%). In 63 of 450 patients no definitive result was obtained by PCR due to simultaneous presence of *S. aureus*, CNS and mecA.
Conclusion: Control of sampling and extraction is very important when performing PCR directly from swabs. In 351 of 450 patients isolation could be stopped due to negative results in PCR for patients at high risk. All proofed positive patients could be isolated. In 14% of all tested patients (PCR indeterminates) isolation for another 2 days until the results of conventional testing was obtained was recommended for patients at high risk. In 86% of all patients with RT-PCR MRSA could be proofed or excluded within 2 h test time instead of 1–3 days by culture.

O64 Evaluation of Multiplex PCR Assay to discriminate *Staphylococcus aureus* from coagulase-negative staphylococci and determine methicillin resistance from culture

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Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) are the major pathogens in nosocomial and community-acquired infections. Molecular methods for the identification of MRSA have shown the need for rapid and dependable methods for the diagnosis of MRSA infectious diseases. In the present study, a multiplex PCR assay was established and evaluated to detect MRSA cells in culture. The new assay targeted both the mecA gene and a 442 base pair DNA fragment (Sa442) within a single PCR.
Methods: Strain representing EMRSA types 1–17 were examined for mecA and Sa442. In addition, 63 strains of MRSA collected from Manchester Royal Infirmary (MRI) were tested. A total of 25 isolates of a number of different species, including Methicillin-sensitive *Staphylococcus aureus* (MSSA), Methicillin resistant *Staphylococcus epidermidis* (MRSE), Methicillin-sensitive *Staphylococcus epidermidis* (MSSE), *Streptococcus pyogenes* and *Escherichia coli* from MRI and an in-house culture collection, were tested to determine the specificity of the assay.
Results: Sa442 primers were found to be specific for *Staphylococcus aureus*, although one of 80 isolates (specificity 98.75%) known to be *Staphylococcus aureus* was negative for Sa442. At the same time, two of 80 (specificity 97.5%) isolates were negative for the mecA gene. These two isolates were phenotypically methicillin resistant. Serial dilutions were done to determine the sensitivity of this method. This method was able to detect as few as 5×102 cells per millilitre.
Conclusion: This multiplex PCR assay represents a rapid and accurate method with potential for direct detection of MRSA from clinical specimens.

O65 Direct detection of vancomycin-resistant enterococci from rectal swabs using automated DNA extraction and rapid real-time PCR: a pilot study

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Objectives: Conventional vancomycin-resistant enterococci (VRE) culture from rectal swabs (RS) can take 5–7 days, decreasing this turn-around-time (TATI is a goal of most laboratories. This study compared direct PCR of VRE in RS to standard and broth enrichment culture methods.
Methods: Each of 209 RS (80 from known VRE carriers in Toronto hospitals; 129 from surveillance in a single hospital) were inoculated using the routine protocol onto m*Enterococcus* vancomycin 6 mg/L agar (EV). Each RS was then rolled in 300 μL sdH_2O for PCR. The RS were then inculated into BHI broth. The BHI was subcultured after 6 h incubation onto Columbia Nalidixic Acid Vancomycin 6 mg/L agar (CNAV). Two vancomycin discs were placed in the main inoculum area on the CNAV. EV were examined daily for 3 days and the CNAV 2 days for PYR + Gram + colonies around the discs. Identification of suspect colonies was performed using broth microdilution and conventional biochemicals. DNA was automatically extracted from the samples after FastPrep disruption and stabilised in STAR buffer using the Total Nucleic Acid Isolation Kit on the MagNa Pure (Roche). PCR for vanA, B,B2/3 genes was by LightCycler (Roche) using the VRE LC Detection Kit (Roche).
Results: A total of 87/209 RS were VRE+ (86 *E. faecium*, one *E. faecalis*; 78 vanA, three vanB, three vanB2/3, three vanA+vanB2/3)by at least one culture method. 64/87 were VRE+ by all three methods. A total of 15/87 did not grow on EV without enrichment (13 vanA, one vanB2/3, one vanA/vanB2/3) and one vanA VRE was detected on EV only. Four of 87 vanA VRE were not detected on CNAV due to overgrowth of Proteus. ≥87 VRE were not detected by PCR. Sensitivities for EV, CNAV and PCR were 85.3, 95.6 and 93.5%, respectively. A total of 122/209 RS were VRE negative:115 were negative by all three methods and initially generated nonrepeatable low-positive readings with melting peaks suggestive of five vanA and two VanB2/3 products. Negative by culture, these seven may represent very low concentrations of VRE but as they were negative on repeat PCR, they were considered false positives, reducing the specificity of PCR to 94.6%. The TAT for PCR, CNAV and EV averaged 6 h, 2–3 days and 4–7 days, respectively.
Conclusions: Direct PCR demonstrated a comparable sensitivity to broth enrichmnet culture while reducing the TAT to within 1 day. Further study is required to assess the impact of VRE PCR testing on a hospital's budget.

O66 Development of DNA microarrays for detection of macrolide-resistance genes

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Objective: Purpose of this study was the development of a microarray-based assay to detect genes encoding resistance to macrolides and related drugs. Such genes are known to cluster within genetic elements; for this reason a complete macolide resistance microarray should be useful as a tool for reliable and fast epidemiological screening.
Methods: A database of resistance genes was constructed by retrieving nonredundant sequences from GenBank. Probes, 40–60 nucleotides in size, were synthesised with a C6-aminolinker and spotted on epoxy-modified glass. The final DNA chip was printed using an Affimetrix GMS 417 robotic spotter, and included a triplicate set of probes, and positive hybridisation controls. Genomic DNA was labelled with Cy3 or Cy5 by random priming. Hybridization signals were then detected using an Affymetrix 418 laser scanner and images were analysed by the GenePix Pro (version 5.0) software.

Results: A total of 109 oligonucleotide probes were designed for recognition of 64 macrolide resistance genes. The DNA chip was tested using 15 control strains from seven different bacterial species (*Staphylococcus aureus*, *Enterococcus faecalis* and *Enterococcus faecium*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus gordonii* and *Escherichia coli*), known to harbour 20 different genes for resistance to macrolides and related drugs. Expected results were obtained with control strains.
Conclusions: We developed a DNA microarray to be used to screen for the presence of macrolide resistance genes in bacteria.

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Helicobacters-update 2004 (Symposium arranged with EHSG)

S80 Sensing *H. pylori* in the stomach

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Helicobacter pylori strains that harbour a pathogenicity island, the 'cagPAI', induce NF-kappaB activation and IL-8 synthesis in gastric epithelial cell lines. The mechanism involved in this process is unknown. Though *H. pylori* is an extracellular bacterium, surface-expressed pathogen recognition molecules (TLR2, TLR4 and TLR5) do not appear to play a significant role in epithelial cell responses to this pathogen. *H. pylori* cagPAI was shown to encode a type IV secretion apparatus, capable of mediating the translocation of effector molecules into host cells. Thus, we speculated that an intracellular molecule may be involved in host sensing of this bacterium. It was recently reported that Nod1/CARD4, a cytosolic molecule with homology to plant resistance proteins, mediated epithelial cell responses to the invasive bacterium, *Shigella flexneri*. These responses were dependent on the recognition of a disaccharide-tripeptide motif present in Gram-negative bacterial peptidoglycan. We showed that *H. pylori* bacteria induced NF-kappaB activation in Nod1-expressing HEK293 cells via a cagPAI-dependent mechanism. Moreover, this response could be abrogated by co-transfection of cells with a Nod1 dominant negative construct, indicating a prime role for Nod1 in epithelial cell signalling to this bacterium. Nod1 was shown to respond to *H. pylori* peptidoglycan that was presented within epithelial cells. We therefore determined the ability of *H. pylori* bacteria to deliver this molecule into host cells. *H. pylori* mutants possessing a nonfunctional cagPAI secretion apparatus delivered significantly reduced levels of 3H-labelled peptidoglycan into epithelial cells. This represents the first example in which a bacterial type IV secretion apparatus has been demonstrated to be engaged in the delivery of a nonprotein effector into host cells. Finally, Nod1-deficient mice were shown to be more susceptible to infection by cagPAI-positive *H. pylori*, when compared with wild-type animals, thus identifying Nod1 as an important factor in innate immune responses to *H. pylori* infection. We suggest that this novel paradigm of pathogen recognition may be applicable to other noninvasive Gram-negative bacteria that cause severe mucosal inflammation in animal hosts.

Issues in smallpox

S87 Europe needs to be prepared

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This paper outlines the urgent measures that were taken from October 2001 onwards by the European Union following the terrorist attacks in the US. It highlights the key elements of the current EU programme of medium and long-term action on chemical, biological and radio-nuclear threats and describes the health security programme which is being implemented since December 2001 and its results. The health security programme forms a key component of the EU action on terrorism. It is now being embedded in a wider effort on emergency preparedness and response that became necessary as a result of the lessons learnt from the SARS epidemic. A Health Security Committee was established in November 2001, a programme of co-operation in the EU on preparedness and response to biological and chemical agent attacks (health security) was drawn up in December 2001 and a Task force on health security was set up with EU experts in May 2002 The programme aims to ensure an EU-wide capability for the timely detection and identification of biological and chemical agents in laboratories, the rapid and reliable determination and diagnosis of relevant human disease cases, the availability of medicines, co-ordination of emergency plans and the drafting and dissemination of rules and guidance on facing-up to attacks from the health point of view. The implementation of the European Union's programme on health security helped to drive action on bioterrorism forward. The future EU Centre for Disease Prevention and Control, proposed by the Commission in July 2003, will be a key player in providing advice to the Member States and the EU institutions, and in implementing surveillance and response actions in the area of health security.

Infections of intravascular devices

S91 Intra-arterial device-related infections

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Objectives: To review clinical aspects of intra-arterial device-related infections and to outline the diagnosis, treatment and prevention of these infections.

Methods: A review of published literature was performed to formulate a summary of intra-arterial device-related infections for oral presentation. Devices and procedures included in this report are peripheral vascular stents and stent-grafts, prosthetic vascular grafts, including those used for haemodialysis, intra-aortic balloon pumps, angioplasty/angioplasty-related bacteraemia, coronary artery stents and carotid patches. The aspects addressed for each

device include epidemiology, microbiology, predisposing risk factors, diagnosis, treatment and prevention.
Results: Risk of infection is variable depending upon the device or procedure. Even for devices that are rarely complicated by infection, it is crucial that a review be done because of the number of devices that are annually implanted on a global basis. Unique clinical findings assist in the diagnosis of infection. Treatment options usually mandate device removal. This often creates a treatment conundrum among patients who are deemed not appropriate surgical candidates for removal of the infected device. Moreover, continued use of the device is necessary to sustain limb or life such that reimplantation of a new device is needed when the infected device is removed. Prevention of infection is a key area of continued investigation. Prevention methods include the use of antimicrobial prophylaxis, alterations in the design and make-up of the devices, implementation of sound infection control practices and immunization.
Conclusions: A understanding of intra-arterial device-related infections is critical because of the ever-increasing number of these devices being placed, the growing number of different types of devices becoming available, and the potential limb- and life-threatening nature of these infections.

Methicillin-resistant *Staphylococcus aureus* – MRSA

O92 Methicillin-resistant *Staphylococcus aureus* (MRSA) reported through the European Antimicrobial Resistance Surveillance System (EARSS), 1999–2002: variation over time and place

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Objectives: We explored the variation in proportions of methicillin-resistant *S. aureus* (MRSA) between and within countries and temporal trends using the antimicrobial susceptibility results of *S. aureus* isolates from blood cultures. From 1999 to 2002, data of 28 countries were reported to the European Antimicrobial Resistance Surveillance System (EARSS).
Methods: Participating laboratories performed routine antimicrobial susceptibility tests for *S. aureus*. Reduced susceptibility to oxacillin was confirmed by quantitative or molecular methods. Data were collected at national level and forwarded to EARSS at the Dutch National Institute of Public Health for further analysis. We analysed data from 495 hospitals that reported results of more than 20 isolates. Time trends were analysed by Poisson regression modelling for 312 hospitals providing data for at least three consecutive years.
Results: EARSS received susceptibility results of 53 264 *S. aureus* blood isolates, of which 50 759 (95%) were included in the current analysis. The prevalence of MRSA differed largely and varied about 100-fold in Europe. A low proportion (<1%) was found in northern Europe and high proportions (>40%) were reported from southern Europe, Ireland, and the UK. Between 1999 and 2002, MRSA proportions significantly increased in Germany (from 9 to 19%), the Netherlands (from 0.4 to 1%), UK (from 31 to 45%), Ireland (from 39 to 45%), and Belgium (from 22 to 27%) and decreased in Slovenia (from 22 to 15%). Within countries, MRSA prevalence varied between hospitals with highest variance in Germany and in other countries with an MRSA prevalence of between 5 and 18%.
Conclusion: MRSA proportions vary substantially between countries and hospitals. Our findings should increase the alertness in countries with increasing MRSA rates. Hospitals with low MRSA prevalence in these countries should maintain their efforts to keep this prevalence low. The occurence of low-prevalence hospitals should also encourage infection control personnel in high-prevalence hospitals to take appropriate measures to contain the MRSA epidemic.

O93 Community-acquired Methicillin-resistant *Staphylococcus aureus*: role of home nursing care in a French prospective study

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Background: Until recently, Methicillin resistant *Staphylococcus aureus* (MRSA) was regarded as a multi-resistant pathogen associated exclusively with nosocomial infections. For 10 years, authors have evoked an epidemiological 'drift' of MRSA towards the community. The question is where exactly MRSA acquisition takes place. The aim of the survey was to research the risk factors in relation to community acquisition of MRSA, concentrating primarily on medical or paramedical care in community.
Methods: A case-control study with prospective recruitment included consecutively 198 cases and 198 controls between the 1st of April 2002 and the 31st of July 2003 in a french teaching hospital. All patients were, at the time of admission, infected with *Staphylococcus aureus*. Cases were infected with MRSA, controls were infected with MSSA.
Findings: Multivariate analysis showed a strong and highly significant independent association between MRSA infection at admission and prior home nursing care (OR = 3.7, $P < 0.0001$). The other independent risk factors were prior hospitalisation in a surgical ward (OR = 3.8, $P = 0.0003$), transfer from another institution (OR = 2.3, $P = 0.008$) and age >65 years (OR = 1.6, $P = 0.04$). In addition, there was a dose-effect relation for prior home nursing care and a positive interaction-effect with prior hospitalisation in a surgical ward (OR = 3.1, $P = 0.02$).
Implications: These results evoke the possibility of true community MRSA acquisitions by home nursing care. The MRSA reservoir are probably MRSA carriers discharged from hospital. A potential vector are domiciliary nurses who, not daily confronted with nosocomial infections and often ignoring the patient status in relation to MRSA, do not apply appropriate hygiene measures.

O94 Carriage of *Staphylococcus aureus* in family units

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Objectives: Although numerous studies have investigated prevalence of nasal carriage of *Staphylococcus aureus*, and several case studies have documented spread within a family, there have been no studies of patterns of carriage in families and risk factors affecting spread in the family. This study aimed to examine family carriage of *S. aureus*.
Methods: One hundred families of students were invited to participate and given an information sheet and consent form. Students were instructed to collect specimens by swabbing the septum adjacent to the nasal ostium. Swabs in transport media were returned to the laboratory within 14 h for culture and isolation of *S. aureus*. Participants provided demographic details and information on contacts with health care, recent antibiotic use and hospitalisation. Isolates of *S. aureus* were screened for methicillin resistance and tested for sensitivity to eight antibiotics. Relatedness of strains was determined by pulsed-field gel electrophoresis (PFGE).
Results: A total of 92 families with 352 individuals participated in the study. Specimens were collected from 332 subjects (94%). The number of family members varied from two to eight (mean 3.8). Seventy-three (22%) subjects from 49 families carried *S. aureus* and there was no significant difference in carriage rates between males and females. Carriage rates were higher in larger families

(greater than three members) ($P \leq 0.001$), and in subjects who had contact with a nursing home (42.8%). Logistic regression indicated that in subjects aged ≥51 years use of antibiotics in the last 4 weeks reduced risk of carriage, whilst long-term treatment increased risk. Methicillin-resistant strains were isolated from three subjects. One subject, who worked in a hospital and had been recently hospitalised, carried a multiply-resistant strain. The other two strains, one isolated from a nursing home employee, were resistant only to beta-lactams. Carriage of MRSA was significantly associated with occupation in health care. PFGE revealed multiple carriers of an identical strain within a family in 10 cases. Different strains were observed in eight families with multiple carriers.
Conclusion: Carriage of *S. aureus* and MRSA were 22 and 0.9%, respectively. Carriage rates were higher in large families and in elderly subjects on long term treatment. PFGE revealed transmission within the family unit. Carriage of MRSA in this community remains low and carriers failed to transmit these strains to other family members.

O95 A group of related EMRSA15 isolates carrying the Panton-Valentine leukocidin gene

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Objectives: To follow-up the discovery of an EMRSA15 isolate carrying the PVL gene, commonly associated with community-acquired MRSA, by searching the Scottish MRSA Reference Laboratory (SMRL) collection of MRSA isolates. Unlike many hospital strains, EMRSA15 usually carries few additional resistances and has the type IV SCCmec element but isolates have not, to our knowledge, been previously reported to carry the PVL gene.
Methods: A clinical microbiologist had drawn to our attention an MRSA isolate from a 6-month-old child who had suffered recurrent infections suggestive of a PVL-producing strain but who was otherwise healthy. This MRSA was shown by PCR to have the PVL gene, the mecA gene and the type-IV SCCmec element; phenotypically it appeared to be an EMRSA15 with an unusual antibiogram. The PFGE-banding pattern of the isolate varied from the commonest EMRSA15 pattern at only one band locus; the change was indicative of an insertion event. We searched the SMRL database for phenotypically and genotypically related isolates, tested them by PCR and further characterised isolates with the PVL gene.
Results: Less than 0.1% of the approximately 30 000 EMRSA15 isolates in our database had the same antibiogram as the index isolate; 21 of these were tested for the PVL gene and 20 were positive. By PFGE, all the PVL-positive isolates had patterns typical of EMRSA15; all had the apparent insertion seen in the index isolate but there was some other variation between the patterns. Most of the PVL-positive isolates had the same, uncommon, phage pattern though three were untypable. The earliest isolate had been received in 1998 and the sending laboratories were from several different geographical regions of Scotland. PCR testing for other toxin genes on five of the isolates has shown that they also carry the enterotoxin C gene, like most other EMRSA15 isolates that we have tested. We have also examined about 60 EMRSA15 isolates identified from our database as similar to the index isolate, but not identical, and all were PVL-negative by PCR; none of the individual recognisable characteristics (susceptibility markers, phage type, PFGE insertion) alone are markers for the PVL gene.
Conclusions: We have shown that a phenotypically identifiable group of EMRSA15 isolates carrying the PVL gene has been circulating in Scotland for at least 5 years.

O96 Increased community-onset MRSA disease prevalence due to MRSA clones colonising the nares of healthy individuals in the community

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Background: The number of MRSA clinical isolates originating from inpatients and outpatients populations has increased more than 3.5-fold between 1996 and 2002 in the San Francisco Community Health Network(CHN) and has been associated with the spread of specific clones.
Objective: A molecular epidemiologic study was undertaken to investigate the underlying causes for this increased MRSA prevalence and a possible association with nasal colonisation.
Methods: The study aimed at assessing temporal changes in MRSA prevalence between two population-based samplings of (i) 833 homeless and marginally housed adults in 1999–2000 and (ii) 308 homeless and runaway youths in 2002. Genotyping by multilocus sequence typing, multilocus restriction fragment typing, pulsed-field gel electrophoresis, and staphylococcal chromosomal cassette mec(SCCmec) was undertaken to profile clonal distribution. The nasal MRSA genotypes were compared with concurrent genotypes recovered from community-onset (CO) MRSA diseases (defined as first culture from clinical sites of patients within 72 h of hospital admission or from an outpatient facility).
Results: MRSA prevalence increased among community nasal carriage, from 2.76% (1.76–4.11, 95% CI) in 1999–2000 to 6.17% (3.75–9.47) in 2002. Over 95% of the nasal MRSA isolates were SCCmec type IV. There was a significant difference in genotype distribution between the time periods. Three genotypes, designated as P, S and Z (ST-59, ST-8 and ST-30)were associated with 80.6% of CO-MRSA disease between 1998 and 2002, these clones were also spreading asymptomatically in the two populations. The P clone was dominant in both 1999–2000 (21.7% of nasal MRSA isolates) and 2002 (42.1%). Concurrent to its spread in the community, the P clone surged in 1999–2002 to account for 20.8% of CO-MRSA disease. That S clone was not found in the community in 1999–2000 matched with its absence among clinical isolates until September 2000. In 2002, S clone accounted for 42.1% of nasal MRSA isolates, corresponding to its surge in prevalence among disease-causing isolates in 2001–2002. The Z clone was widespread among CO-MRSA diseases in 1999, however, only one Z nasal isolate was found.This lack of spreading through asymptomatic nasal carriage in the community by Z clone explains its drop in prevalence in disease in 2001–2002.
Conclusion: Nasal colonisation by certain MRSA clones is correlated with widepread MRSA disease originating from the community.

Non-molecular diagnostic methods

O97 External quality assessment for microbiology – participant performance with anaerobes

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Objectives: To assess the results from clinical diagnostic laboratories taking part in the UK National Quality Assessment Service general bacteriology scheme with specimens containing anaerobes, and to compare performance with repeat specimens.
Methods: Quality assessment of general bacteriology ($n = 800$; 328 UK, 472 overseas) for the isolation and identification of bacterial pathogens, was performed on anaerobic organisms including *Actinomyces israelii*, *Actinomyces odontolyticus*, *Bacteriodes fragilis*, *Clostridium perfringens*, *Clostridium tetani*, *Clostridium novyi* type A, *Clostridium septicum*, *Fusobacterium necrophorum*, *Peptostreptococcus anaerobius*, *Porphyromonas endontalis* and *Prevotella intermedia*.
Results: The overall level of performance with specimens containing anaerobes over the last 10 years was 76% of participants'

reporting the correct result; which compares with 93% for all identification specimens in the general bacteriology scheme over the same period. Participants' performance with *Clostridium* species was on average a 78% success rate within a range of 65% for a *C. tetani* to >90% for specimens containing *C. perfringens*, *C. septicum* and *C. novyi*. Performance data for other anaerobes varied considerably and showed a 97% success rate for a specimen containing *Peptostreptococcus anaerobius*, 52% for a *Prevotella intermedia* and 56% for a *Fusobacterium necrophorum*. Analysis of results for anaerobes distributed more than once showed that with *Bacteroides fragilis* performance was consistent with success rates of 84 and 80% over two specimens. In contrast, over five specimens of *Actinomyces israelii* the success rate was 86, 86, 59, 60 and 81%, respectively.

Conclusions: These data provide an interesting insight into overall performance with anaerobes. The level of performance seen with some anaerobes such as *Clostridium* species was generally better than with others, such as *Prevotella* or *Bacteroides* species. Performance over time with repeat specimens was variable, and no significant improvement was seen with any anaerobe. Although the level of identification of anaerobes required to influence patient management is the subject of debate among microbiologists, the failure to isolate anaerobes should be of concern, and fully investigated by the laboratories concerned.

O98 Microbial approach in diagnosis of infection in orthopaedic implants

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Objective: Orthopaedic device-related infections are rare, but they carry a high morbidity for patients and are costly. Replacement of total joint prosthesis is the traditional choice in management therapy of aseptic and septic loosing of implants. Factors influencing success are a high-level of experience of the orthopaedic surgeon, the isolation of pathogens and suspectibility pattern for postoperative therapy. Standard culture techniques can often be moderately sensitive, because bacteria colonising the surface of implants grow predominantly in adherent biofilms. We aimed to improve the isolation of bacteria from hip prostheses removed by using sonication.

Methods: From March 2001 to September 2003 we retrieved 109 hip prostheses from patients at revision operations for aseptic loosening 61× or septic loosening 48× of implants. The femoral and acetabular components after sonication and tissue contact with the implants after homogenisation in Ringer solution were cultured aerobically and anaerobically. The routine specimen, swab and aspirate of joint fluid, were cultured aerobically and anaerobically simultaneously. The organisms isolated were identified by biochemical API BioMerieux system and sensitivity to antibiotics was tested by standard laboratory methods.

Results: Cultures (49.2%) of the aseptic loosing were positive. Components were positive in 96.7%. Components and tissue in 53.3%, components, tissue and routine specimen were positive in 20%. Bacterial strains isolated: coagulase-negative staphylococci in 70.5%, streptococci in 18.2%, *Propionibacterium* sp. in 11.3%. In 87.5% cultures of septic loosing were positive. Components were positive in 83.3% of the cultures, components and tissue were positive in 61.9% and components, tissue and routine specimen were positive in 40.5%. Bacterial strains isolated: coagulase-negative staphylococci in 31.3%, streptococci in 37.5%, *Staphylococcus aureus* in 14.6%, *Propionibacterium* sp. in 8.3%, enterobacterie and *Pseudomonas* sp. in 8.3%.

Conclusions: We believe that sonication technique may increase detection of infection of total hip replacement because sonication increases sensitivity of the culture technique by dispersing adherent bacteria which were not isolated by the routine cultures.

O99 Rapid diagnosis of leptospirosis: identification and use of a 25 amino-acid peptide [PP (R)] from the haemolysin-associated protein Hap1

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Leptospirosis is an important infectious disease of humans and animals worldwide. Diagnosis is often difficult because of symptoms ranging from subclinical infection to a severe multi-organ involvement leading to death in five to 15% of the cases. Early and accurate diagnosis of leptospirosis is essential for proper treatment, which is lifesaving for patient. Up to now, MAT is the gold standard but is late, laborious and time consuming. There is an urgent need for development of new diagnostic strategies for leptospirosis and particularly in the initial phase of the disease.

Objectives: Our previous work determined that the haemolysis-associated protein Hap1 (as known LipL32), able to induce a protection against leptospire challenge, is expressed in the early phase of leptospirosis infection by only pathogenic leptospires. ELISA against the recombinant protein Hap1 is very sensitive but not enough specific. To avoid this lack of specificity, we identified and characterized an antigenic epitope of the Hap1 protein. Then, we used this peptide as antigen ELISA against a panel of human sera.

Methods: Based on analysis of hydrophobicity and conformation of the Hap1 protein, we selected antigenic region and synthesized three peptides of possible epitopes. The synthetic peptides were screened and the PP peptide was identified. Then, sera from human, vaccinated or not against leptospirosis, postinfectious of confirmed leptospirosis or Lyme disease were analysed by PP-ELISA (IgM and IgG) and MAT.

Results: One of the three peptides is recognised by monoclonal antibody against rHap1 and positive control anti-leptospire sera. This peptide was used as antigen in ELISA to test more than 150 human sera. The peptide PP, highly conserved part of the protein Hap1, is early recognising during the first step of leptospirosis infection. The results demonstrated a good agreement between the PP-ELISA and MAT results in leptospirosis cases.

Conclusion: The results of this study show that PP-ELISA is a very sensitive and specific tool for diagnosis of leptospirosis. Contrary to MAT, this serological test allows differentiating of vaccinal or postinfectious antibody. These finding indicate that the peptide PP may be a useful antigen for rapid serodiagnosis of leptospirosis.

O100 ELISA-based rapid diagnosis of Rift Valley fever

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Objectives: Recent outbreaks of Rift Valley fever (RVF) in humans and livestock outside the African region resulted in an increased international demand for laboratory diagnosis of this zoonotic disease. Disadvantages of the classical methods include health risk to laboratory personnel and inability to distinguish between diffrent classes of immunoglobulins. Therefore, our aim was to develop and validate IgM-capture, IgG-sandwich and antigen-capture ELISA for rapid and safe diagnosis of RVF.

Methods: In house produced biologicals were gamma-irradiated, safety tested and freeze-dried before use. Development and validation of assays followed standard laboratory and statistical procedures. The virus neutralisation test (VNT) and infectivity assay in cell cultures were used for the verification of infection status with RVF virus. To determine the diagnostic accuracy of IgM and IgG ELISA human sera collected during outbreaks of RVF in Africa, diagnostic submissions, and sera from laboratory personnel vaccinated against RVF, were used. Cut-off values for IgM and IgG ELISA were optimised using various statistical approaches including the two-graph receiver operating characteristic analysis. Detection limit for antigen-capture ELISA was initially estimated by titration of sucrose-gradient purified RVF virus,

and then verified using results from laboratory generated and experimental specimens, and infected mosquito pools collected in the field during the 2000 RVF outbreak in the Arabian Peninsula.
Results: There was no evidence for excessive variations within and between runs of the assays. Based on our present data the specificity of IgM ELISA was 99.4%; about 80% of VNT-positive sera collected during active RVF outbreaks tested positive. The specificity of IgG ELISA was 99.2% and sensitivity 100%. The detection limit of antigen-capture ELISA was approximately 2000 TCID50/mL. There was no evidence for cross-reactions with common African phleboviruses, and the ELISA easily detected RVF virus isolates from disparate historical and geographical origins.
Conclusions: As safe, robust and highly accurate methods, the ELISAs have the potential to replace the traditional diagnostic assays, which pose a biohazard and thus have to be limited to high containment facilities. Potential applications are: early diagnosis of infection, monitoring of immune responses in vaccines, epidemiological surveillance of humans, livestock and vector populations.

O101 A study to assess the efficacy of a MALDI-TOF-MS database for the rapid identification of *Bacillus* isolates

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The genus *Bacillus* is immensely complex and is currently undergoing major taxonomic revisions based mainly upon 16S rDNA sequence analysis. Traditionally species are identified using morphological and biochemical criteria such as crystal formation on PEMBA agar and lecithinase production. However, these tests can be prolonged and may not conclusively allow species-level identification.

MALDI-TOF-MS is a rapid novel technique that allows the surface components of bacterial cells to be resolved as a characteristic mass spectrum. A small amount of growth from a culture plate is applied to a target plate and mixed with matrix solution. Irradiation of the sample with a nitrogen laser in a mass spectrometer produces a plume of ions that are determined using time of flight mass spectrometry.

Using a linear M@LDI instrument, (Waters Corporation, Manchester, UK), this technique was applied to National Collection of Type Culture (NCTC) strains representing *Bacillus* food pathogens frequently received for identification in the Food Safety Microbiology Laboratory (FSML), HPA, London. These included *B. subtilis*, *B. cereus*, *B. megaterium*, *B. sphaericus*, *B. pumilus* and *B. lichenformis*. Using cluster analysis MALDI-TOF-MS analysis grouped the data into species-specific clusters.

Subsequently a dedicated MALDI-TOF database (Manchester Metropolitan University, Manchester, UK) consisting of >2000 entries and representing nearly 90 strains of Bacillus was utilised in combination with database matching software (Waters Corporation, Manchester, UK) to analyse 80 well-characterised clinical isolates from FSML, HPA, London. All *B. subtilis* isolates were correctly identified and 75 and 79% *B. cereus* and *B. lichenformis* isolates, respectively were successfully identified with high percentage probability matches. Nine isolates of *B. pumilus* were incorrectly identified, but upon close inspection of the data biomarkers that enabled successful differentiation of *B. pumilus* from these organisms was located. In conclusion, these preliminary findings suggest that MALDI-TOF-MS will represent a useful tool for the rapid identification of *Bacillus* isolates.

Pathogenesis of infection

O102 Participation of gut commensal bacteria and mucosal immunity in pathogenesis of inflammatory bowel disease (IBD): gnotobiotic models of human disease

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Objectives: Immune system associated with mucosal surfaces covering the largest area of the body (200–300 m) evolved mechanisms discriminating between harmless commensal microorganisms and dangerous pathogens. Numerous inflammatory and autoimmune diseases may occur as a result of changes in mechanisms regulating mucosal immunity, which leads to impaired barrier function of the mucosa, increased penetration of components of commensal flora and/or food into circulation and consequently to abberant immune responses. The aim of our study was to elucidate the initial events leading to development of intestinal inflammation in animal models of human IBD (i.e. Crohn's diseases and ulcerative colitis) reared in gnotobiotic conditions.
Methods: We analysed the role of commensal bacteria in pathogenesis of gut inflammation using experimental model of colitis developing spontaneously in colon of conventional SCID mice (mice with severe combined immunodeficiency) reconstituted with the CD45RBhigh subset of CD4+ T cells isolated from spleen of normal BALB/c mice. As a recipients, germfree SCID mice, conventional SCID mice or ex-germfree SCID mice monoassociated with defined bacterial strains or colonised with bacterial mixtures were used. Mice were colonised with segmented filamentous bacteria (SFB) alone or with defined bacterial cocktail of specific pathogen-free (SPF) mice.
Results: Clinical picture and histopathological evaluation revealed that 8–12 weeks after transfer of pathogenic CD4+CD45RBhigh T cell subpopulation into germfree SCID recipients intestinal inflammation did not develop. In contrast, severe forms of intestinal inflammation were present in conventionally reared SCID mice and in mice colonised with cocktail of SPF microflora plus segmented filamentous bacteria (SFB). A monoassociation of SCID mice with SFB, *Escherichia coli*, *Enterococcus fecalis*, *Bacteroides distasonis* or various cocktails of SPF bacteria did not lead to intestinal inflammation. Signs of impairment in tight junctions (TJ) of epithelial cell layer in the terminal ileum of colitic mice was documented by anti ZO-1 antibody.
Conclusion: Our studies suggest that noncultivable segmented filamentous bacteria together with defined mixture of cultivable bacteria from SPF mice were effective in triggering intestinal inflammation. Supported by EU project QLRT-CT-1999–0050.

O103 Identification and disruption of opuB, a novel bile tolerance locus linked to the virulence potential of *Listeria monocytogenes*

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Listeria monocytogenes is the causative agent of listeriosis, a debilitating illness accounting for approximately 35% of all deaths caused by known bacterial foodborne pathogens in the US yearly. *Listeria* is capable of withstanding a variety of hostile environmental conditions, including the many stresses encountered during the production, preparation and storage of food and postconsumption within the animal host. The ability of *Listeria* to proliferate under such adverse conditions is attributed, at least in part, to the accumulation of compatible solutes (of which betaine, carnitine and choline are most effective), and the active extrusion of harmful compounds such as toxic bile salts produced by the host.
Objective: To mine the listerial genome sequence for potential membrane transporters, which accumulate beneficial compounds

or extrude harmful ones, thereby allowing the pathogen to mount a successful infection.
Methods and results: *In silico* analysis of the *L. monocytogenes* genome revealed a fused two-gene operon designated opuB, exhibiting significant sequence similarity to certain members of the betaine carnitine choline transporter (BCCT) family. Preceded by consensus sigA and sigB-dependent promoter-binding sites we demonstrate by reverse transcription PCR analysis that the operon is transcriptionally up-regulated at elevated osmolarities and reduced temperatures (stresses known to induce sigB). Furthermore, a significant reduction in the level of opuB transcription was observed in the absence of sigB. In addition, we identified an important role for PrfA, the master regulator of virulence potential in *L. monocytogenes*, in coordinating opuB expression. Computer-aided structural analysis of the OpuB protein suggests that, rather than functioning as a compatible solute uptake system, OpuB is in fact an efflux pump, actively extruding bile salts from inside the cell, a finding confirmed by radiolabelled bile efflux studies. In addition, functionally inactivating OpuB by chromosomal exchange mutagenesis resulted in an approximately 4 log decrease in the ability of the bacterium to tolerate bile, a phenotype which translates to a significant reduction in virulence potential when administered to a murine model by the oral route.
Conclusion: OpuB, which is coordinately regulated by sigB and PrfA, represents a novel bile tolerance locus in *L. monocytogenes*, which contributes significantly to the virulence potential of the pathogen following infection via the oral route.

O104 The impact of mechanical ventilation on the treatment with moxifloxacin (MXF) in an animal model of pneumonia caused by *Streptococcus pneumoniae*

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Objective: *Streptococcus pneumoniae* is a leading cause of community-acquired pneumonia (CAP) worldwide. About 25% of hospitalized patients with CAP need intensive care unit management. In this setting, pneumonia is still associated with a mortality of 30–46% in spite of the availability of effective antibiotic therapy, especially when mechanical ventilation (MV) is required. Experimental data suggest that MV could reduce lung bacterial clearance. Therefore we studied in which extent MV could influence the efficacy of MXF, a new active antibiotic treatment against *S. pneumoniae*.
Methods: Prospective experimental study in NZ healthy rabbits ($n = 71$). A calibrated inoculum of *S. pneumoniae* (MIC for penicillin, ciprofloxacin and MXF were 4, 0.5 and 0.125 mg/L respectively) was intrabronchially instillated in all the animals. Four hours later, a human-like treatment of MXF corresponding to the standard regimen (400 mg i.v. od for 48 h) was initiated in animals undergoing MV and in air-breathing ones. Either mechanically ventilated or air-breathing untreated rabbits were used as controls. Survivors were sacrificed 48 h after inoculation. Pneumonia assessment was based on histologic (macroscopic score) and bacteriologic (lung and spleen cultures results) findings.
Results: Whereas MXF treatment was associated with a substantial survival improvement in the air-breathing animals (21.6% vs. 100% of survivors, in control and treated animals, respectively), mortality rate remained high in the presence of MV (12.5% vs. 46.1%). In the presence of MV, pneumonia tended to be more severe in terms of histologic damage. The lung bacterial burden was higher in treated animals with MV compared with those without (5.1±2.4 vs. 1.6±1.4 log 10 CFU/g, respectively; $P < 0.05$). Pneumonia was bacteraemic in almost all the untreated animals. No animals was bacteraemic in the air-breathing animals treated with MXF. In contrast, 23.1% of the treated animals had positive spleen cultures in the presence of MV. Notably, MXF blood concentrations was found to be comparable in both groups.
Conclusions: In our model of *S. pneumoniae* pneumonia, MV seemed to worsen lung injury and to promote bacterial growth and systemic spread of the infection. In such conditions, MXF was less effective than in air-breathing animals. Taken together, these results suggest that MV may impair host lung defence, making less-effective antibiotic therapy.

O105 The cylA/B genes of the *Streptococcus agalactiae* haemolysin genecluster encode a multidrug resistance ABC transporter

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Objectives: The *Streptococcus agalactiae* haemolysin is regarded as an important virulence factor. Haemolysin production is dependent on the cyl genecluster. This cluster comprises 12 genes and was identified in 1999 by our group through the analysis of non-haemolytic and nonpigmented ISS1 mutants. cylA and cylB encode the ATP-binding and transmembrane domains of a typical ABC transporter.
Methods and results: The deduced proteins from cylA and cylB display significant homologies to multidrug resistance (MDR) transporters. Insertion mutants in this locus result in a nonhaemolytic and nonpigmented phenotype. The function of these genes was further elucidated by construction of nonpolar deletion mutants of cylA. These mutants are nonhaemolytic and can be partially complemented by a plasmid harbouring cylA and cylB. To identify substrates of the putative transporter the wildtype strain, a cylA and a cylK deletion mutant were exposed to known substrates of MDR transporters. While the susceptibility of the mutants to various antibiotics was unchanged, growth of the cylA deletion mutants was significantly impaired in the presence of 0.5 mg/L daunorubicin and 1 mg/L doxorubicin. However, the growth pattern of the nonhaemolytic cylK deletion mutant was indistinguishable from the wildtype strain. This indicates that the observed effect is linked to the transporter activity and not to the nonhaemolytic phenotype. Further indications for the importance of the transporter to get haemolysis is supported by the fact that addition of the multidrug pump inhibitor reserpine to culture media markedly reduced the haemolytic activity.
Conclusion: To our knowledge this is the first report of a *Streptococcus agalactiae* transporter with the ability to transport substances such as daunorubicin and doxorubicin. This finding along with the reserpine inhibition of haemolytic activity may provide some insight into the chemical structure of possible natural substrates of cylA/B.

O106 Resorbable hydrogel polymer as an antimicrobial delivery system in the treatment of *Staphylococcus aureus* experimental osteomyelitis

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Purpose: Polymethylmethacrylate bone cement (PMMA) is used for antimicrobial delivery in treatment of bone infections. Antimicrobial release from PMMA is limited and PMMA beads used for local antibiotic delivery need surgical removal after treatment. Resorbable vancomycin-loaded hydrogel polymer beads (RPH) will release most of the vancomycin loaded and can be resorbed *in situ*. We determined the release kinetics of vancomycin from RPH and PMMA beads in a continuous flow chamber and used a rat model of *S. aureus* osteomyelitis to compare the activity of RPH and PMMA delivered vancomycin.
Methods: Vancomycin concentration was measured in the effluent of a flow chamber containing one 3 mm RPH or PMMA bead containing 7.5% vancomycin. The effluent was sampled every 2 h for 48 h. Results were expressed as the mean peak concentration, mean AUC 0–48 h and mean per cent of antibiotic released for RPH or PMMA. Experimental osteomyelitis was established in

Wistar rats by inoculating a sclerosing agent and 10^6 *S. aureus* into the medullary cavity of the proximal left tibia. 4 weeks after infecting the rats, treatment by debridement of the infection site and placement of a bead in the bone defect was initiated. After 3 weeks of treatment, rats were sacrificed and the tibia removed and cultured. The results were expressed as the median log 10 CFU/g of bone.
Results: *In vitro* release kinetics of vancomycin from RPH or PMMA are: RPH peak concentration 62.7 μg/mL, AUC 508 h $\times$ μg/mL and 56% of vancomycin loaded into beads was released. PMMA peak concentration 19.1 μg/mL, AUC 180 h $\times$ μg/mL and 11% of vancomycin loaded into beads was released. *In vivo* results of treatment in *S. aureus* experimental osteomyelitis are listed as median log 10 CFU/g (range 25–75th percentile). Results of treatment with debridement alone were 6.01 CFU/g (5.1–6.5), bland PMMA 6.4 CFU/g (6.1–6.8), bland RPH 5.9 CFU/g (4.4–6.3), PMMA delivered vancomycin 3.2 CFU/g (1.5–4.1) and RPH-delivered vancomycin 4.5 CFU/g (3.9–6.0).
Conclusion: The vancomycin peak concentration, AUC (0–48 h) and the per cent released into physiological buffer in our continuous flow chamber were greater from RPH than PMMA. In the treatment of MRSA experimental osteomyelitis, vancomycin loaded RPH beads are more active ($P < 0.05$) than debridement alone, bland RPH or PMMA bead but less active ($P = 0.02$) than vancomycin-loaded PMMA beads.

Pan-European infectious disease surveillance: do we get the needed information for public health action?

S108 Early warning of influenza epidemics: the EISS network

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The European Influenza Surveillance Scheme (EISS) was established in 1996 and is a pan-European influenza surveillance network that covers 22 countries and 25 surveillance networks. The scheme includes 30 National Reference Laboratories, over 11 000 sentinel physicians and covers a total population of 445 million inhabitants (http://www.eiss.org). EISS is funded by the European Commission (EC) and aims to integrate all of the European Union Member States. The aim of the EISS project is to reduce morbidity and mortality associated with influenza in Europe. EISS monitors influenza by collecting clinical and virological data on a weekly basis during the influenza season from two sources: sentinel physicians (clinical and virological data) and virological data from other sources (e.g. hospitals and nonsentinel physicians). During the influenza season, EISS publishes a Weekly Electronic Bulletin on its website, which provides an epidemiological and virological overview of influenza activity in Europe. EISS acts as an early warning system for influenza by collecting and presenting detailed data on a timely basis to its members and the general public. During the 2002–2003 season, EISS monitored the emergence of the new drift variant A/Fujian/411/2002 (H3N2) at the end of the season and it has monitored the spread and impact of this virus during the 2003–2004 season. Members share their data and expertise with the group and EISS makes public health statements concerning new viruses or public health threats (e.g. a statement for the EC on the impact of the A/Fujian-like virus). In the context of the EC's Community Influenza Pandemic Preparedness and Response Plan, EISS has established a Community Network of National Reference Laboratories for Human Influenza in Europe. This initiative will lead to improved collaborations between laboratories, the use of standard WHO reagents and the establishment of an accreditation system for National Reference Laboratories. In connection with pandemic preparedness activities, EISS is also initiating contacts with surveillance schemes that monitor influenza in animals. These developments should, in turn, enhance the surveillance of influenza in Europe and improve the early warning functions of EISS to detect influenza epidemics and a possible pandemic.

S109 The EU-MenNet: understanding the changing patterns of meningococcal disease

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Meningococcal disease remains a major childhood infection in Europe, with an appreciable number of cases in other age groups, notably young adults. There is currently no comprehensive childhood vaccine against this disease, the severity of which, combined with its rapid progression and nonspecific symptoms, results in an unacceptable burden of childhood morbidity and mortality. The development of effective vaccines and public health management policies are confounded by the epidemiology of meningococcal disease, which is itself governed by the complex population biology of the causative organism, *Neisseria meningitidis*. In addition to its intrinsic relevance for European public health, meningococcal disease is a valuable scientific model system and a paradigm for pan-European co-operation among publically funded laboratories. In 2001 the EU-MenNet consortium representing all European reference laboratories for meningococci has been established to address the need for harmonized nucleic acid sequence-based detection and typing technologies and the implementation of the multi-locus sequence typing (MLST) technology throughout Europe. The molecular strain characterisation data together with integrated epidemiology information gathered by this consortium provides substantial added value in terms of understanding the dynamics of spread of hypervirulent meningococcal clonal complexes, which are responsible for almost all cases of invasive meningococcal disease. It will now be possible to develop pan-European recommendations for the management of this important disease and to compare the impact of different vaccine strategies and help to select the best vaccination schedules. These technologies will also be applicable to future meningococcal serogroup B vaccines.

S110 EWGLINET: towards legionella-free travel in Europe

C. Joseph – European Working Group for Legionella Infections

Objectives: To identify, control and prevent outbreaks of Legionnaires' disease in European travellers.
Methods: Since 1987, European microbiologists and epidemiologists have collaborated in EWGLINET – a scheme for the identification of travel associated legionella cases, rapid detection of clusters linked to tourist accommodation and exchange of information so that immediate investigations and control measures can be implemented. Collaborators also contribute to improving clinical and environmental methods for case and outbreak confirmation and to enhancing national surveillance schemes for case detection. In July 2002 European Guidelines for the Control and Prevention of Travel Associated Legionnaires' Disease were introduced. They aim to standardise procedures for informing and liaising with hotels, tour operators, public health officials and the general public, and for carrying out investigations when clusters occur. They were endorsed by the EC in June 2003 and are now used by the majority of European countries. Sanctions are applied if the country of infection fails to implement the required public health response in the time frame set out in the Guidelines. These sanctions include publishing information about the cluster and the accommodation site on the EWGLI website. Clusters are also publicised if public health measures are considered to be inadequate for control and prevention of further cases.

Results: A total of 36 countries now participate in EWGLINET. Cases reported to the scheme have increased from three in 1987 to 168 in 1995 and 675 in 2002. Highest numbers in 2002 were linked to Italy (132), France (122), Spain (85), Turkey (81) and Greece (33). How countries of infection respond to single cases influences the number of sites that are associated with further cases. In Italy, France and Spain, between 18 and 21% of their total cases were linked to clusters whereas in Greece and Turkey it was much higher at 36 and 59%, respectively. In total 94 clusters were detected in 2002, 27 of them in Turkey. Dealing with the clusters has been slow in Turkey, and 78% of implicated sites were published on the EWGLI website between July 2002 and October 2003.
Conclusions: Management of clusters in most countries is progressing well through use of the Guidelines. The next challenge is to apply greater efforts to improve prevention of legionella. This paper will review how this work is contributing towards the objective of legionella-free travel in Europe.

The threat posed by Rickettsiales and other arthropod-borne pathogens (Symposium arranged with ESCAR)

S111 Climate impacts on ticks as vectors

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Pathogens transmitted by vectors are faced with ecological and epidemiological hurdles defined by the quantitative balance between all the factors that determine the rates of transmission. These depend to a large extent by the impact of a variety of extrinsic environmental factors on the vector–host–pathogen triangle. Of the three types of organisms within this triangle the vector is the most sensitive to climate. Using the framework of a simple tick population model, I shall present field data and experimental results that help to quantify the relationships between climatic conditions and tick demographic rates and therefore pathogen-transmission rates. Different rates may change in different directions in response to climatic variation. For example, tick development rates increase with increasing temperature, but tick host-seeking activity (and therefore feeding rates and long-term survival) decreases with increasing moisture stress. Climate may even influence the differential attachment of each tick stage to different host species, particularly to rodents that are commonly important transmission hosts. The outcome in terms of pathogen-transmission potential can only be predicted with a fully functional model. This is especially true for any pathogen, such as tick-borne encephalitis virus, that requires certain patterns of tick seasonal dynamics to support the specific cellular basis of transmission by ticks via rodents. Nevertheless, until we have a full biological process-based model, we can use a statistical approach to predict the likely impact of forecast climate change on the distribution of vector-borne diseases.

S112 Prophylaxis for tick-borne diseases

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To date ticks are the main arthropod-vectors for human disease in developed countries. They can transmit a broad number of tick-borne pathogens (bacterial, virus, protozoa, toxins) with a high rate of morbidity. Tick-borne diseases prophylaxis can be made by means of pre-exposition measures or after exposition ones. Among pre-exposition measures is very important to wear correct clothes when we go to the possible tick-infected areas (long trousers inside the socks, boots, shirt with long sleeves, and a cap or similar). The colour of the clothes is also important, so the dark ones attract less ticks than bright ones, but in the last ones we can see them better and remove them before they can bite us. The use of repellents like permithrin in clothes is safe for health although short in time but not very effective in my experience. Other important measure is the removing of the parasites of our pets to avoid introducing ticks at home. A measure to reduce ticks is controlling rodents population and vegetation at town surrounding areas. Since in Lyme borreliosis has been demonstrated that the tick must feed on his host during 24–48 h, it's very important to look for ticks when we come back from tick-infested areas. So if we remove ticks before they transmit borrelias we can prevent Lyme borreliosis. This measure is less important for preventing Rickettsiosis or Ehrlichiosis since these bacteria can be transmitted precociously. After tick-bite there are two important questions. How must I remove the tick? Must I take antibiotics for prophylaxis? Ticks must be removed with forceps. Other removal methods as oil, petrol, cigarette, etc, should be avoided. In reference to the second question, only an American study has demonstrated that the use of a single dose of 200 mg of doxiciclyne is effective for preventing Lyme borreliosis. In Europe none study has been conducted for answering this question. By other hand this measure has not been evaluated for preventing Rickettsiosis and other tick-borne diseases. Vaccines for preventing tick-borne encephalitis (Centro-European encephalitis) are safe and are indicated in endemic areas. Lyme borreliosis vaccines were used in USA and took out from market because of adverse effects.

Hepatitis C virus infection: pathogenesis, diagnosis and treatment

S116 Humoral response against hepatitis C virus

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Antibodies are often a reliable marker indicating vigorous response against infectious agents and in several viral diseases presence of anti-viral antibodies indicates an effective protection. However, in the case of hepatitis C virus (HCV) in spite of an intense antibody response there is no protection against a new infection and often the virus overcomes host defences establishing a persistent infection. There is a lively debate about whether the humoral response affords any protection against HCV in the infected host. Indeed, whereas some studies have shown that a high titre of anti-HCV antibodies does not prevent reinfection, several other reports indicate the presence of a protective response consistently with the demonstration that immunoglobulins may prevent the infection in patients at risk. Finally, description of the dynamics of intra-host evolution have shown that a crucial phase for disease outcome lies at a time point correspond-

ing to the production of antibodies by the infected host. These data suggest an important role for antibodies in the evolution of HCV infection. An important structure studied as a target of antibody is HCV E2 envelope glycoprotein (HCV/E2), but unfortunately the assessment of the efficacy of this class of antibodies has been hampered by the poor growth efficiency of HCV in cell culture, by the fact that the murine response is not consistent with the human antibody response and by the unavailability of animal models of this infection beside primates. Cloning of the immune repertoire of an HCV-infected patient on phage display combinatorial vectors and generation of human monoclonal antibodies have demonstrated that activity varies widely for single clones. In particular efficiency in neutralizing HCV/E2 binding to cellular target and in inhibiting infection of cells by HCV pseudoviruses is different for each clone. The observation that only some clones are able to inhibit HCV, can help understand how this virus escapes the control of the immune system. A better characterization of the anti-HCV immune response in terms of biological activity will help the design of possible vaccine strategies and their *in vitro* evaluation. This would be a considerable advance in an infection lacking an animal model. Molecules demonstrated *in vitro* to stimulate selectively the production of neutralizing antibody clones will be the best candidates for further *in vivo* studies.

Multidrug resistance in *Acinetobacter*, *Stenotrophomonas* and *Pseudomonas*: similarities and differences – interactive session

S124 Epidemiology of multidrug resistance in *Acinetobacter, Stenotrophomonas* and *Pseudomonas*

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The three well-known nonfermentative organisms, namely *Pseudomonas aeruginosa, Acinetobacter* spp, and *Stenotrophomonas maltophilia* are common commensals and ubiquitous organisms widely distributed in nature. In addition to factors involved in their virulences, resistance to a wide variety of antimicrobials makes these organisms effective opportunistic nosocomial pathogens. Although resistance patterns may differ in these bacteria, multidrug-resistant (MDR) strains are usually not affected by all betalactams including carbapenems, aminoglycosides, and quinolones. In epidemiological studies, respiratory tract was found the most frequent site of isolation for all three and patients residing in the ICU were more frequently infected. *P. aeruginosa* was reported as the second most common cause of nosocomial pneumonia by NNIS system, whereas *Acinetobacter* was the seventh for this infection in a large European surveillance study. *S. maltophilia* has been increasingly reported as a cause of infection in immunosuppressed patients, however its incidence was far less common than the previous ones. In a recent SENTRY trial the incidence of MDR *P. aeruginosa* was reported 4.7% in European centers. The rate was increased up to 20% in certain Italian, Portuguese and Turkish centers. In general the highest rates were found in centres from South America and this was closely followed by centers from Europe. Increasing carbapenem resistance worldwide is a big concern in both *P. aeruginosa* and *Acinetobacter* isolates and is becoming a disturbing clinical problem. Against MDR *P. aeruginosa* and *Acinetobacter* isolates, the most effective agents are polymyxins, however resistance to these agents has already been described in *Acinetobacter* isolates. Similarly, resistance exists for the most-effective agents against *S. maltophilia*, namely trimethoprim-sulphamethoxazole (upto 10%), ticarcillin-clavulanate (10–29%), and quinolones (up to 15%). Epidemic occurences with these three nonfermentatives possessing MDR capabilities seem to be increasing. Ongoing surveillance for isolates and their resistance profiles is important to guide therapy.

HIV: from research to clinical facts

O127 Complement synthesis and activation in the brain of SIV-infected monkeys

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Objectives: Complement is one of the most important defence tools against cerebral infections, but uncontrolled complement biosynthesis and activation can induce brain inflammation and profound tissue damage. To clarify the role of complement in AIDS-associated neurological disorders we analysed complement synthesis in the brains of SIV-infected rhesus macaques.
Methods: Immunohistochemical staining in paraffin-embedded tissue sections derived from the brain of SIV-infected rhesus macaques and uninfected control monkeys was performed using specific antibodies against the complement factors C1q and C3.
Results: The cerebral synthesis of complement factors C1q and C3 is strongly upregulated in SIV-infected monkeys compared with the spontaneous synthesis in the brain of uninfected control animals. Astrocytes, neurons, microglia, and infiltrating macrophages and multinuclear giant cells all contribute to the high amounts of C1q and C3 present in the SIV-infected brains. Secreted C1q and C3 are not only present extracellularly in the tissue, but are also deposited on the membrane of neurons, a prerequisite for formation of the membrane-driven lytic membrane attack complex.
Conclusion: The low constitutive production of complement in the normal monkey brain is highly upregulated by infection with SIV, indicating that enhancement of complement synthesis is an important defence mechanism of the brain against invading virions. The membrane deposition of complement on brain cells, however, suggest a role of complement and complement-induced lysis of bystander neurons as a potential mechanism for HIV-induced cell damage.

O128 HE2000 decreases inflammatory cytokines and viral load in HIV patients and opportunistic infections in late stage AIDS patients

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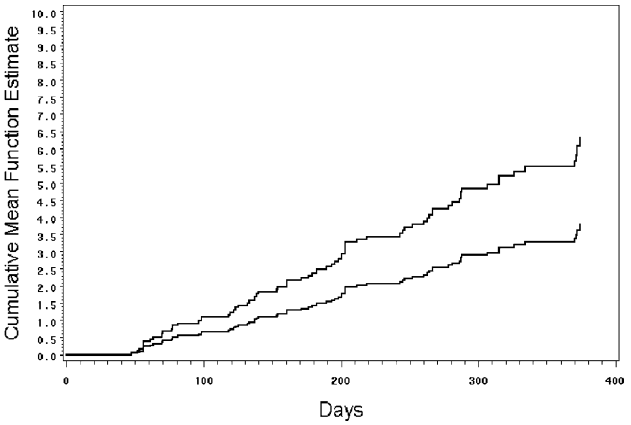
Objectives: HE2000, 16-α-bromoepiandrosterone, is a broad spectrum immune therapeutic agent that has demonstrated preclinical

and clinical activity in HIV/AIDS models and patients. Clinical studies were conducted to determine the effect of HE2000 on the incidence of opportunistic infections in late stage AIDS patients. The capacity of HE2000 to act as a therapeutic vaccine to stimulate T-cell HIV-specific immunity in HIV patients was also examined.

Methods: Two double-blinded placebo-controlled clinical studies were conducted in South Africa in treatment naive HIV patients(CD4 > 200) and AIDS patients (CD4 < 100) at risk for opportunistic infections. Patients were cyclically treated with HE2000 once daily for 5 days every 6 weeks.

Results: In the study of HIV patients (baseline mean CD4 cells = 475 for treated and 429 for placebo groups), immune modulation occurred after two to three cycles of treatment. When compared with placebo, HE2000 normalized inflammatory cytokines (TNF-α and IL1-β), increased circulating dentritic cells, stimulated gag-specific T-cell immunity and lowered viral titre 0.6 log ($P = 0.01$). In late stage AIDS patients (treated group baseline mean CD4 = 49.9), a statistical trend ($P = 0.10$) in eduction of C-reactive protein levels was noted compared with the placebo group (baseline mean CD4 = 55). Viral load decreased by 0.55 log using an area under the curve minus baseline model (AUCMB). The data is suggestive of an increase in CD4 cells ($P = 0.11$ NS). There was a delay in the median time to the first emergent tuberculosis infection (>406 days) when compared with the placebo (281 days). A statistically significant decrease in the cumulative incidence of opportunistic infections was observed in the treated group ($P = 0.027$).



Conclusions: HE2000 restores HIV-specific immunity in HIV patients, which results in a decrease in viral load and it decreases total cumulative opportunistic infections in late stage AIDS patients. HE2000 may delay the need for antiretroviral therapy in the treatment of HIV infection.

O129 Differential effect of HAART on CD4 and CD8 T-cell cytokine production and response to CMV antigens in HIV+ patients

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Objectives: To determine the effect of HAART on cytokine production and response to CMV antigens in CD4+ and CD8+ T-cells in HIV+ patients no on anti-retroviral therapy and following initiation of HAART.

Methods: A total of 73 patients with HIV infection were divided into those stable without HAART and those on HAART. Patients on HAART were divided further on the basis of their length of time on HAART (> or <3 months), viral load (> or <50 copies/mL, CD4 count nadir and whether their CD4 count had increased to >150 cells/μL on HAART, gender and origin. CD4+ and CD8+ T-lymphocyte cytokine production in response to stimulation with PMA and ionomycin using single cell flow cytometry was performed. In a further cohort of 104 CMV+/HIV+ patients on HAART, proliferative and cytokine responses to CMV antigens were analysed and compared.

Results: CD4+ and CD8+ IFNg expression was increased in HIV+ groups when compared with controls. CD4+ cell IFNg expression normalized on HAART when CD4 count was >150 cells/μL. CD4+ IL-2 expression was significantly reduced in all HIV patients when compared with controls and did not significantly improve on HAART. However IL-2 production by CD8 cells was significantly improved in all patients on HAART when compared with HIV patients on no HAART. This was normalized in patients following HAART for 3 months or greater, when their CD4 count exceeded 150 cells/μL or when the HIV viral load was <50 copies/mL. In response to CMV antigens, all groups showed a significant reduction in CD4+ T-cell function and production of cytokines, despite an increase in absolute CD4+ T-cell numbers. By contrast in patients responding to HAART, CMV-specific CD8+ T-cells have increased responses to CMV.

Conclusion: These results show that the production of IL-2 by CD8 cells and IFNg by CD4+ cells becomes normalized on successful HAART and that even at low CD4+ counts CD8+ responses to CMV are enhanced. This is likely to account for the early clinical improvement in rate of opportunistic infections and reduced risk of death in patients on HAART.

O130 DAPY non-nucleoside inhibitors of HIV-1 reverse transcriptase possess high activity in microbicide assays and have potential for prevention of sexual transmission of HIV

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Objectives: Several diaryl-pyrimidine (DAPY) compounds have nanomolar activity in cell-based assays on wild type and mutant HIV-1. They bind into the non-nucleoside site of HIV-1 reverse transcriptase (NNRT). Three DAPY compounds are presently in clinical studies. In parallel, microbicide assays have been carried out on DAPYs in order to assess their potential for prevention of transmission of HIV.

Methods: In a first set of experiments, monocyte-derived dendritic and autologous CD4+ T cells (MO-DC/T) were infected with HIV-1 Ba-L, washed and treated for 14 days with the test compound, yielding the 50% effective concentration (EC50). In a second set, exposure to compound was 24 h. After 24 h, cells were washed and co-cultured for 14 days without compound, followed by secondary culture with PHA/IL-2 blasts. In a third set, virus was immobilized on poly-L-lysine and pretreated with compound for 1 h in the absence of cells. After washing, MO-DC/T were added and cultured during 14 days.

Results: EC50s of the DAPYs in the 14-days experiment ranged between 0.4 and 3 nM. For comparison, EC50s for UC-781 and PMPA in this test were 53 and 106 nM, respectively. Dapivirine (TMC120-R147681), the prototype DAPY, showed only a small increase in EC50 in the 24-h assay and completely protected against HIV between 10 and 100 nM, whereas 100-fold higher concentrations were required with UC-781 and PMPA. In the 1-h assay, dapivirine completely inhibited replication between 10 and 100 nM, while UC-781 required 100-fold higher concentrations.

Conclusion: NNRTIs of the DAPY-class of compounds are highly potent inhibitors of HIV-1 replication in microbicide assays which mimick conditions of sexual transmission of HIV. DAPYs, such as dapivirine, may possess virucidal activity against HIV in the absence of target cells. DAPY NNRTIs present high potential for development as microbicides in order to prevent sexual transmission of HIV, using vehicles such as gels and intravaginal rings.

O131 Q151M complex is frequent in HIV-1 non-B subtype isolates of patients living in southern Spain

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Introduction and objective: Certain polymorphisms in the pol gene of HIV-1 have been found more frequently in non-B HIV-1 subtypes. As this may be important in optimising antirretroviral therapy we studied the prevalence of pol mutations and related them to HIV subtype in a cohort of HIV-1 patients living in the south of Spain.
Patients and methods: We studied 292 HIV-1 patients. Sequencing of the pol gene was made by Trugene HIV-1 genotyping Kit (Bayer). Subtyping was performed with the pol sequence using phylogenetic analysis and reference sequences from B and non-B strains (Stanford HIV-1 database and ABL networks). SPSS 11.0 package was used for statistical analysis.
Results: Subtyping is expressed for protease/reverse transcriptase genes. A total of 280 patients harboured B subtypes (B/B) as studied in the pol gene of HIV-1. The rest were as follows: three F1/B, two D/B, one CRF02_AG, one G/CRF02_AG, one C (C/C), one G (G/G); for one patient only the RT sequence was available and it was classified as K subtype. For RT gene, Q151M mutation alone, the mutations of its complex, and K219Q were detected more frequently in patients infected with non-B subtypes ($P < 0.001$); L210W, was detected with a higher prevalence in patients infected with B subtypes ($P < 0.001$). For the protease gene, K20M and M36I were more prevalent when patients were infected with a non-B subtype. No differences in the antirretroviral regimen were observed among patients infected with B and non-B subtypes.
Conclusions: Multi-nRTI resistance Q151M complex is more frequently detected in patients infected with non-B HIV-1 subtypes. This is of extremely importance when selecting antiretroviral regimens for patients infected with non-B subtypes, and strengthens the importance of HIV-1 subtyping information as a part of the resistance report.

O132 Comparison of two algorithms in the interpretation of HIV-1 drug resistance genotypic data and a novel phenotypic assay, virtual phenotype

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Objective: The aim of this work was to assess the concordance of two different genotypic resistance interpretation algorithms and the recently introduce phenotypic assay, Virtual Phenotype.
Material and methods: A collection of 105 plasma samples with viral load >1000 HIV-1 RNA copies/mL (Roche Amplicor Monitor Assay) was included in the study. Viral RNA extracted (TRUPREPTM Extraction Kit, Visible Genetics) was analysed genotypically using TRUGENE HIV-1 Genotyping Kit assay according to the manufacturer's instructions (Visible Genetics). Sixteen antiretroviral drugs were analysed. Results were interpreted with either Visible Genetics version 5 and 6 (VG), RetrogramTM 1.4. (RG) and Virtual Phenotype (Virco Inc's) (VP). The algorithms report different levels of resistance, so to obtain output normalisation interpretations, no evidence of resistance 'S' form VG has been compared with RG class A, resistant 'R' from VG to RG class D and possible resistance 'I' from VG to RG class B and C. To compare with VP we considered susceptible, resistant and an intermediate level, when >60%, <40% and the 40–60% of the strains were within normal susceptible range, respectively . The three algorithms were compared between them grouped into three pairs, concordance analysis between the different interpretation systems was conducted by kappa measurement.
Results: The algorithms assigned 1526 drug resistance interpretations to 105 sequences. Complete concordance results among the three algorithms were found in the 59.1% of the samples. 27.8% were assigned an S, 29.4% an R and 1.9% an I level. The percentage of minor discordances (S/I and R/I) was 32.4% and major discordances (S/R) were found for 8.39%. In pair-wise comparisons, low concordances in the interpretation was observed for most nucleoside reverse transcriptase inhibitors, mainly tenofovir, didanosine, zalcitabin and abacavir. Results agreed highly for all nonnucleoside reverse transcriptase inhibitors and most protease inhibitors.
Conclusions: (i) There exists a great level of discordance in the interpretation of genotyping results among algorithms mainly for nucleoside reverse transcriptase inhibitors. (ii) A consensus for the interpretation of genotypic data and clinical validation of genotypic results are needed. (iii). Prospective comparative studies are needed taking into account the broad spectrum of factors that can affect the clinical outcome.

O133 Surgical site infections in HIV-infected patients from the MASTER cohort

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Objective: The aim of this study was (i) to evaluate the incidence of surgical site infection (SSI) in a cohort of HIV-infected patients (HIV+) and (ii) to identify risk factors associated with SSI in HIV+ patients.
Materials and methods: We carried out a retrospective study involving HIV+ patients seen at the HIV clinic in Brescia, Italy. Each patient was asked about surgical interventions performed since they knew they were HIV+. We recorded data regarding: (i) surgical intervention: type and duration of intervention, ASA score, level wound contamination, type and duration of antibiotic prophylaxis; (ii) HIV infection: preoperative and postoperative CD4 cell count, plasma HIV-1 viraemia, antiretroviral treatment (ART), if present; (iii) known factors for SSI: neutrophil count, length of preoperative hospitalization, smoking habit, body-mass index (BMI), diabetes mellitus, presence of an active infection, treatment with steroids. We also recorded the risk index category as reported by the National Nosocomial Infection Surveillance System (NNISS). The SSI rate was compared with that reported from the NNISS, matched for intervention type.
Results: Over a period of 2 months, we interviewed 829 HIV+ patients: 229 (27.6%) reported at least one surgical procedure. A total of 46 clinical charts (20%) were available for analysis. The interventions were of general surgery (31%), orthopaedic (24%) and gynaecological (22%). The number of surgical procedures increased over the last 5 years, with a significant difference between the period 1985 and 1997 and 1998 and 2003 ($P < 0.001$). The median age was 40 years (interquartile variation (IVQ): 35–46) and 72% were males. The CD4 cell count was $323/mm^3$ (175–727), and HIV viraemia was 67 (IVQ 50–1350) copies/mcl, 82% of patients were on ART. A SSI was diagnosed in three (6.5%) of the 46 evaluable patients: in one case superficial and in two cases, deep. The rate of SSI was three times higher than that reported by the NNIS system for matched interventions. No statistical differences was identified between the patients with and those without SSI, regarding the evaluated parameters related to SSI risk, probably due to the small number of patients.
Conclusions: Although the number of intervention analysed is quite small, this study shows that: (i) there is an increase in the number of HIV+ patients who undergo surgery; (ii) the rate of SSI in this group is very high. Specific systems to reduce the risk of infection should be searched for HIV-infected subjects.

O134 Dyslipidaemia during HIV disease: a comparison of efavirenz- and nevirapine-based regimens

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Objective: To assess the serum lipid safety profile of the two available non-nucleoside reverse transcriptase inhibitors (NNRTI): efavirenz (E) and nevirapine (N).
Methods: A cross-sectional study performed on 988 HIV-infected patients (p) treated with antiretrovirals during >12 months and naïve to NNRTI,allowed us to identify 234 p given E, and 212 p who introduced N. After excluding 47 p due to compliance <90%, 208 p who received E were compared with 191 p treated with N, by a multivariate analysis focusing on adverse events, toxicity, and related treatment interruptions.
Results: Study groups were comparable as to demographic–epidemiologic features, HIV disease stage, mean HIV viraemia and mean CD4+ lymphocyte count, rate of HCV and/or HBV co-infection, antiretroviral therapy background, and pre-existing metabolic disturbances and/or lipodistrophy syndrome (in pretreated p). When examining the 121 p naïve to antiretrovirals, the tolerability index measured during the first 3 months of therapy did not differ between the two NNRTI, but clinical features were substantially different, with predominant hypersensitivity reactions for N, and CNS disturbances for E ($P < 0.0001$). When considering p experienced with antiretrovirals, and p on salvage regimens, a grade 1–3 liver toxicity occurred in >20% of N-treated p, vs. three p only in the E group ($P < 0.0001$). When a NNRTI substituted a protease inhibitor due to prior dysmetabolism, a drop of triglyceridaemia and/or cholesterolaemia >30% vs. time of NNRTI introduction, occurred in over 66% of p who switched towards N, compared with <30% of those who introduced E ($P < 0.0001$), while in 10 p a frank dyslipidaemia appeared only after E use.
Conclusion: The two available NNRTI have a comparable activity and resistance profile, but the remarkably different pattern of potential adverse events has to be carefully considered, due to the broad spectrum of short- and long-term toxicity, and the significant differences between E and N, in terms of incidence and clinical presentation of untoward events. A long-term observation of p pretreated with other anti-HIV regimens seems to show a tendency towards a cumulative liver toxicity for N, and stable or worsening metabolic (and lipid) abnormalities for E. The potential pathogenetic pathways of the remarkably different toxicity patterns of these two compounds belonging to the same therapeutic class, warrants enlarged investigation.

O135 Comparison between basal parameters in hepatitis due to HCV alone and HCV-HIV co-infected patients. A different spectrum of the same disease

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Objective: Hepatitis C virus (HCV) is currently the most important cause of chronic liver disease and liver-related death. HCV–HIV co-infection is the most frequently diagnosed associated pathology in the natural history of HIV-infected patients in the first world, even more than opportunistic infection. Quantitative determination of HCV RNA viral load and HCV viral genotype in baseline sera are standarised methods for evaluation of HVC-infected patients. Plasma HCV viraemia and HCV genotype are considered predictive for response to treatment. The aim of this study was to evaluate and compare the levels of basal HCV RNA in HCV mono-infected patients and HCV–HIV co-infected patients, and the genotype distribution in both groups of patients.
Methods: A longitudinal observational case-control (1:1) study including the consecutive new and untreated HCV-infected patients attended in a hepatology service were selected. Similarly, we analysed the consecutive new and untreated HIV–HCV co-infected patients visited in the HIV unit. Quantitative serum HCV RNA and HCV genotype were done in all patients using PCR techniques by Amplicor Monitor. The genotype was analysed using hybridisation with specific probes of the different genotypes after PCR.
Results: A total of 146 HCV-infected patients were included in this study, 73 of them were HIV co-infected; 93 (64.3%) were man. The mean age was 42.78 + 7.8 years (28–77). The mean RNA VHC viral load was 1.357.467 + 1.737.139 UI/mL (4.940 + 12.200.000). HCV genotype were 1:86 (59.7%), 2:5 (3.4%), 3:29 (20.1%), 4:24 (16.7%). The mean RNA HCV viral load was 453.598 UI/mL + 274.121 and 2.248.954 UI/mL + 2.082.892 for HCV alone and co-infected patients, respectively ($P < 0.001$). Genotype data were (HCV alone: HCV/HIV) 1:69% vs. 50%, 2:6.9% vs. 0%, 3:15.3% vs. 25.0%, 4:8.3% vs. 25.0%, ($P < 0.001$). HCV viral load (UI/mL) was 474.936 and 2.519.927 ($P < 0.001$), 380.400 and 0 (no evaluable), 461.581 and 2.570.772 ($P < 0.019$), 322.150 and 1.353.463 ($P < 0.001$) for genotype 1, 2, 3 and 4 for HCV alone and co-infected patients, respectively.
Conclusions: Basal HCV RNA viral load was significantly higher in coinfected. We found stastistical significantly differences between both groups with respect of genotypes. Significant differences were found between both groups of patients when comparing viral load for different genotypes.

O136 Is *Legionella* a coincidental or opportunistic infection in HIV-positive patients?

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Objectives: Bacterial pneumonia is, at present, the most prevalent and severe HIV-associated bacterial infection. The microorganisms most frequently involved in community-acquired pneumonia (CAP) are *S. pneumoniae* and *H. influenzae*, especially since the initiation of HAART and successful prophylaxis against *P. carinii*. *Legionella* is currently the second cause of CAP in general population but its incidence is, unexpectedly, not high in this subset of patients. From 1983 to 2003, 18 cases of Legionnaires' diseases (LD) in HIV patients were prospectively attended in our centre. Clinical and immunological data were analysed.
Methods: Data related to HIV and *Legionella* infection were prospectivelly collected.
Results: All the cases were caused by *L. pneumophila*. *L. pneumophila* sg one urinary antigen (LUA) was positive in 13 (72.2%), 6 (33.3%) seroconverted and 1 (5.5%) had a positive broncoalveolar lavage culture. Most of the patients (77.8%) were males with a mean age of 38.4 years (25–62). LD was hospital-acquired in five (27.8%). Three (18.8%) were on TMP/SMX prophylaxis. Eleven (64.7%) were on HAART. The mean CD4 count was 348.1 (6–889). Seven (53.8%) had an undetectable viral load. Fifteen (83.3%) received appropriate antibiotic treatment (macrolides or fluoroquinolones) following admission. Thirteen (72.2%) were smokers and 5 (27.7%) had neoplasms. Fifteen (93.8%) had cough and nine (56.3%) expectoration. Five (31.3%) had chest pain and 12 (75%) dyspnea. Extrarespiratory symptoms were present in 10 (62.5%). Thirteen (76.5%) had an increase in AST, nine (56.3%) hyponatraemia and eight (50%) an increase in CK. Coinfection with other microorganisms could not be demonstrated in any of the patients. Fifteen (83.3%) developed respiratory failure, nine (50%) had bilateral x-ray infiltrates and the mortality was 22.2%.

Conclusions: Legionnaires' disease in HIV-infected patients may be considered an opportunistic infection because of the high rate of respiratory failure, bilateral infiltrates and mortality observed despite adequate antibiotic treatment.

Community-acquired infections: alert to unusual epidemics and update on *S. pneumonia*

O137 Prevalence, phenotypes, genotypes and molecular epidemiology of macrolide-resistant clinical isolates of *Streptococcus pneumoniae* in Norway, 2001–2002

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Background: We have limited knowledge regarding the prevalence and molecular epidemiology of macrolide resistant *Streptococcus pneumoniae* in Norway.
Objectives: (i) Examine the prevalence of macrolide resistance in clinical *S. pneumoniae*-isolates in Norway. (ii) Characterize macrolide-resistant *S. pneumoniae* strains with regard to resistance phenotype and genotype and serotype and multilocus sequence typing (MLST).
Methods: A total of 1708 isolates of *S. pneumoniae* isolates from blood cultures (invasive), respiratory tract and wound specimens (noninvasive), were collected in Norway in two periods in 2001–2002 by 24 participating laboratories within the NORM surveillance programme for antimicrobial resistance in human pathogens. Strains were examined for their susceptibility to erythromycin by E-test. Reduced susceptibility (MIC > 1 mg/L) was detected in 55 (3.2%) *S. pneumoniae* isolates. Further analyses included by E-tests analysis against azithromycin, chloramphenicol, ciprofloxacin, clindamycin, erythromycin, penicillin G, tetracycline, trimethroprim-sulpha and double-disk-diffusion (erythromycin and clindamycin) and serotyping and MLST. Genotypic analyses were performed by erm- and mef-specific PCRs.
Results: A total of 35 (64%) *S. pneumoniae* isolates demonstrated M phenotype resistance, and all harboured mef(A) gene. The remaining 19 (35%) isolates constitutively expressed MLSB resistance and all but one carried the erm(B) gene. One strain (ML) was reproducible negative in erm and mef PCR analyses, and will be further investigated. Reduced susceptibility to penicillin was found in 14 (26%) isolates. These strains were also resistant to tetracycline and erm(B) positive. Serotypes 14, 6 and 19F accounted for 45, 23 and 15% of the strains, respectively. Serotype 14, confirmed as sequence type (ST) nine by MLST, dominated among the invasive strains (67%). Among the noninvasive isolates, serotype 19F was the most common (31%), of which two strains were ST230.
Conclusions: (i) The prevalence of macrolide resistance among clinical *S. pneumoniae* isolates in Norway is still low. (ii) The M-type of resistance was the most prevalent (64%). (iii) Co-resistance to penicillin and tetracycline was common and linked to MLSB resistance-erm(B). (iv) A clonal spread of serotype 14 possessing mef(A), characterized by MLST as ST9, was detected in invasive strains.

O138 Trends in resistance of *Streptococcus pneumoniae* in Europe: an EARSS study

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Objectives: *Streptococcus pneumoniae* (SPN) is an important cause of otitis media and invasive infections. Of particular concern is the high incidence of pneumococcal infections with antibiotic-resistant strains. The present study describes resistance trends for penicillin and erythromycin among invasive SPN isolates and forecasts future trends in Europe.
Methods: The European Antimicrobial Resistance Surveillance System (EARSS) has collected routine antimicrobial susceptibility test (AST) results of primary invasive SPN isolates from blood or cerebrospinal fluid (CSF) since 1999. The proportion of resistance is described as the per cent of SPN isolates only resistant (nonsusceptible) to penicillin, only resistant to erythromycin, or resistant to both. To observe and predict changes of resistance over time a multinomial logistic regression model was used. Seven European countries (BE, DE, FI, IE, IT, SE and UK) that reported more than 100 isolates per year for the period 1999–2002 were included (total number of SPN isolates = 10.989).
Results: Overall, the highest proportion of resistance was observed for the strains only resistant to erythromycin, which increased from 14.6% in 1999 to 16.8% in 2002 (rate of increase = 1.059/year) and is predicted to increase further to 20.4% by 2006. The proportion of strains co-resistant to erythromycin and penicillin increased from 5.4% in 1999 to 6.6% in 2002, and is predicted to increase to 8.9% by 2006 (rate of increase = 1.076/year). A decrease of 4.8 to 4.4% over time (1999–2002) was observed for the proportion strains only nonsusceptible to penicillin, and is predicted to be 3.6% by 2006 (rate of decrease = 0.947/year).
Conclusions: Erythromycin resistance in SPN has exceeded penicillin resistance and is predicted to increase even further in the future, with or without combined resistance to penicillin. The increase of co-resistant SPN strains and the decrease of strains only resistant to penicillin, indicates a shift of only penicillin resistance to co-resistance with erythromycin. This increasing trend of erythromycin resistance emphasises (i) the need for prudent use of macrolides, (ii) the need to encourage the use of high doses of β-lactam antibiotics, especially when isolates are susceptible or intermediately resistant to penicillins and (iii) the importance to introduce vaccination to prevent pneumococcal infections.

O139 Changing patterns of pneumococcal (SP) carriage after an intervention programme

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Background: Naso-pharyngeal SP carriage was studied among children attending day-care centres (DCC) during Winters 1999 and 2002 in Northern (N) and South-eastern France (AM). An intervention programme to promote judicious antibiotic use started in AM in 2000. Trends in serotype distribution and penicillin-resistance (PDSP) were compared.
Methods: NP aspirates were obtained from a random sample of children attending DCC in 1999 and 2002 in N and AM. SP serotypes (6B, 9V, 14, 19A, 19F, 23F and NT) and penicillin susceptibility were determined. The intervention programme included peer-conducted academic detailing visits to all AM general practitioners and paediatricians and parent information.
Results: In 1999 SP serotype distribution and carriage rates, and PDSP/SP for each serotype were comparable in both areas. In 2002, SP carriage was higher ($P = 0.01$) and PDSP/SP was lower ($P < 10^{-4}$) in AM. Only three children in N and 14 in AM had

received pneumococcal conjugate vaccine. Changes in serotype distribution are shown:

	N					AM				
	1999		2002		*P*	1999		2002		*P*
N	250	%	240	%		290	%	294	%	
SP isoloates	117	48.8	117	48.8	ns	161	54.0	174	59.2	ns
PDSP/SP	84	71.8	100	85.5	0.01	102	63.4	112	64.4	ns
Serotypes										
19A	10	8.5	8	6.8	ns	2	1.2	10	5.7	0.02
23F	30	25.6	32	27.4	ns	50	31.1	29	16.7	0.002
PDSP/SP										
6B	18/33	54.5	23/25	92.0	0.001	17/33	51.5	26/35	74.3	0.051
19F	6/10	60.0	16/16	100.0	0.01	7/9	36.8	18/26	69.2	0.03
23F	29/30	96.7	31/32	96.9	ns	49/50	98.0	24/29	82.8	0.02

Conclusion: In 2002, resistance rates increased in N and not in AM; 23F strains were more often susceptible in AM. Proportion of PDSP among 6B strains increased in N, as did 19F strains in both areas. Differences observed between N and AM are encouraging and in favour of pursuing the intervention programme.

O140 Increasing prevalence of multiresistant *S. pneumoniae* in Europe over a 10-year period: Alexander Project

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Objective: To contrast *S. pneumoniae* resistance rates to single or multiple antibiotics over a 10-year period in a global resistance surveillance study, the Alexander Project.
Methods: Isolates were collected from centres in France, Germany, Spain, Italy and the UK annually during the period 1992–2001. MICs and resistance to erythromycin (ERY-R), penicillin (PEN-R), doxycycline (DOX-R) and cotrimoxazole (COT-R) were determined each year according to NCCLS methodology.
Results: ERY resistance in the period 1992–2001 followed a pattern of high and low resistance countries, where France, Italy and Spain had resistance rates that increased steadily from the low teens in 1992 to 56, 36 and 26% in 2001 respectively; and Germany and the UK maintained low levels of macrolide resistance, increasing slowly to a level of 7.5 and 11.5% in 2001. The pattern remains the same regarding COT-R, since it increased from below 10% in 1992 in France, Spain and Italy to 29, 46 and 26% in 2001, respectively. Germany and the UK had COT-R rates that peaked in 1998 and then declined to reach 5.4 and 6.9% in 2001. Resistance to PEN has a similar pattern, being in 2001 <5% in the UK, Italy and Germany, and 36 and 30% in France and Spain. In 1992 there was no joint ERY-COT resistance in the UK, Germany and Italy; levels for France and Spain were 1.8 and 7.8%, respectively. By 2001 joint ERY-COT resistance had increased to 25, 19 and 12% in France, Spain and Italy while remaining under 3% in Germany and the UK. In the case of France, COT-R resistance represented 7% of all ERY-R isolates in 1992, while in 2001 that figure was 6.3 times higher or 44%. Joint PEN-ERY resistance in 2001 had significant levels only in France and Spain, 33 and 17%. The only country that had triple resistance ERY-COT-DOX in 1992 was Spain (6.7%), with France and Spain having levels above 3% in 2001 (14.4 and 17%). Of all ERY-R isolates in Spain, 64% were triple resistant in 2001. Interestingly the levels of joint PEN-ERY-COT-DOX-R were almost the same in France and Spain as those of triple resistance (14.4 and 16%).
Conclusions: Multidrug-resistant pneumococci are an increasingly common finding in Europe. Three out of four ERY-R isolates are also resistant to PEN, COT and DOX in Spain. The rate of growth of multidrug resistance is higher than that of single antibiotic resistance and is increasing by approximately 3% per year.

O141 Comparison of the clonality of penicillin and macrolide non-susceptible pneumococci

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Objectives: *Streptococcus pneumoniae* is still the most important respiratory pathogen. The penicillin resistance is mainly caused by alterations of penicillin-binding proteins, often a consequence of mosaic gene formation. However, the main resistance mechanism for the macrolides is either chemical modification or active efflux pump. The genes causing these are usually located on mobile genetic elements. We examined how these differences influence the clonality of the isolates and the spread of resistance.
Methods: Of an original cohort of 304 Hungarian pneumococcal isolates, collected in 2000–2002, we examined the 112 penicillin nonsusceptible (PNS) and the 82 macrolide nonsusceptible (MNS) strains in the study ($n = 154$). The identity of the strains was confirmed by optochin sensitivity and by the presence of the lytA gene. Serotyping was done with the agglutination antisera by Mast. PFGE was performed with ApaI enzyme and the patterns analysed by the Bionumerics programme.
Results: Isolates of serotypes 9 and 23 very genetically closely related, both belonging to only a single PFGE type. This means that the resistance is spreading among these strains from one generation to the other, originated from a common ancestor some time ago. These were only PNS (second column). We could prove that these strains are closely related to the internationally widespread clones Spain9V-3 and Spain23F-1, respectively, both being macrolide sensitive clones.However, the isolates of serotypes 6 and 19 showed a much bigger genetic diversity (<85% identity). This is possibly because these strains are predominantly macrolide resistant, where the resistance is transferred horizontally by acquiring the resistance genes erm or mef, largely on transposons, to strains that have different genetic background. Interestingly the isolates of serotype 14 were also very clonal, although they usually have high-level macrolide resistance with erm(B).

Serotype distribution (%) and colonality of the strains

Serotype	$P^{NS}M^{S}$	$P^{S}M^{NS}$	$P^{NS}M^{S}$	All P^{NS}	All M^{NS}
6	26	55	45	33	50
9	32	–	–	21	–
14	8	7	20	13	13
19	3	26	23	10	24
23	25	–	–	16	–
Total no.	72	42	40	112	82
	Clonal	Not clonal	Clonal		

Conclusion: Penicillin-resistance in penumococci is spread clonally, whereas macrolide-resistance is much more diverse.

O142 Twenty-five years of tularaemia in Norway, 1978–2002

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Objectives: Tularaemia is a zoonotic disease caused by *Francisella tularensis*, a small aerobic, Gram-negative coccoid bacter-

ium. *F. tularensis* biovar palearctica found in Europe generally causes milder disease than the North American variant, *F tularensis* biovar tularensis. Tularaemia was first described in Norway in 1929 and has been a mandatory notifiable disease since 1975. The neighbouring countries of Sweden and Finland have reported large outbreaks of tularaemia, with mosquito bites as probable mode of transmission. The objective of this study was to describe the epidemiology of tularaemia in Norway.

Methods: The electronic register of the Norwegian surveillance system for communicable diseases was used to identify and analyse reported cases of tularaemia from 1978–2002.

Results: A total of 155 cases were reported from 18 of the 19 counties with peaks at 3–4 years intervals. The geographical distribution was uneven with 111 cases (72%) being infected in four adjoining counties (Sør-Trøndelag, Nord-Trøndelag, Nordland and Troms) in Middle and Northern Norway, comprising only 17% of the country's population. Ninety-five patients (61%) were male. Mean age was 37.6 years, (range 0–79). Disease occurred in all calendar months with no clear seasonal pattern. More detailed information was available for the period 1994–2002: A probable source of infection could be determined in 32 of 50 cases based on individual records and outbreak settings. In 24 cases (75%) infection was due to contaminated drinking water, six (19%) were due to insect or tick-bite and two (6%) were due to direct contact with a rodent. Sufficient clinical detail was reported in 34 cases to determine the clinical manifestation of tularaemia. Pharyngeal tularaemia accounted for 24 (71%), ulceroglandular/glandular tularaemia for nine (26%) and ocular tularaemia for one case (3%). Forty-five cases were diagnosed by serological tests, two cases by serology and culture, one case by culture alone and two cases by PCR.

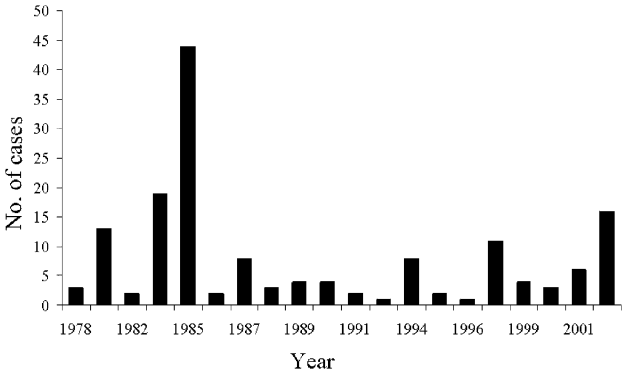

Figure 1.

Conclusions: Tularaemia is endemic in Norway with outbreaks occurring at intervals of several years, often in years with high rodent populations. Contaminated drinking water appears to be the dominating mode of transmission, explaining why oropharyngeal tularaemia is the most common disease manifestation. In contrast to Sweden and Finland, only a few cases could be attributed to insect or tick-bite. Most cases were diagnosed by serological tests.

O143 Cluster of tetanus cases associated with injecting drug misuse in England

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Objectives: To present the clinical features and management of a patient with severe tetanus, and the epidemiological investigation of a recent cluster of cases of tetanus in injecting drug users (IDU) in England.

Methods and Results: A 40-year-old male IDU was admitted to an orthopaedic ward in Liverpool in November 2003 with an acute injection-related abscess in his forearm. Within 24 h, he developed muscle stiffness in the affected arm, difficulty swallowing then trismus with risus sardonicus, opisthotonus, blepharospasm and respiratory spasms (video to be shown). He had no recent tetanus immunizations and had recently changed his heroin supplier. A clinical diagnosis of tetanus was made and he was treated with i.v. penicillin, metronidazole and anti-tetanus serum before surgical debridement of the abscess which yielded staphylococci but not *Clostridium tetani*. He required assisted ventilation for 21 days, complicated by dramatic fluctuations of pulse and blood pressure, but eventually recovered.

This was the second of nine cases of tetanus in IDU reported in England in 2003, the first in July and the remainder in October–December. The cluster was initially identified by astute microbiologists and clinicians liaising with their public health colleagues. Six of nine were female with median (range) ages 32 years (20–47). Seven patients required ventilation and one died. Only two could remember any recent tetanus immunization and at least four had pretreatment serum tetanus antitoxin levels below the minimum protective level (<0.1 IU/mL). It is presumed that one or more contaminated batches of heroin were the vehicles of infection.

Conclusions: (i) Tetanus associated with drug injection has been recognised since the late 1800s and still had a high mortality in New York in the 1960s and 1970s. Mortality is lower with modern intensive care. (ii) Tetanus should be considered in patients who are 'stiff' and/or who cannot swallow. (iii) Health prevention measures for IDU should include checking that tetanus immunisations are complete.

O144 The impact of antibiotic therapy on cognitive and emotional changes among neurobrucellosis patients

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Objective: The aim of this study is to determine the cognitive and emotional changes in patients with neurobrucellosis and the impact of the antibiotic therapy on these changes .

Methods: Patients, who had been hospitalised between 2002 and 2003 in the Infectious Diseases and Clinical Microbiology Department of Ankara Numune Education and Research Hospital, were evaluated prospectively.

Neurobrucellosis was diagnosed by the following criteria; (i) symptoms or clinical findings compatible with neurobrucellosis, including headache, confusion, mental and emotional changes, (ii) isolation of *Brucella* spp. from CSF and/or demonstration of antibodies to *Brucella* >1/4 in the CSF and/or (iii) the presence of lymphocytosis, increased protein and decreased glucose levels in the CSF and (iv) clinical improvement with appropriate treatment. The study was performed in collaboration with psychiatry department of the hospital. Two psychiatrists interviewed the patients, and performed Hamilton Rating Scale for Depression (HAM) and Mini-Mental State Examination (MMSE) to the neurobrucellosis patients at the admission day before the therapy and at the end of first and second weeks of the therapy. The score between 7–14 in HAM with 17 items is accepted as mild depression and scores <25 in MMSE is accepted as decreased cognitive functions. No anti-depressive and no anti-psychotic drugs were given to the patients. Paired *t*-test was performed to compare the test results in different periods (STATA 8.0, USA).

Results: Twenty-nine neurobrucellosis cases were included, the mean age was 41 years, 41% were female. The most common professions were farmers (46%), housewives (25%), workers (7%). At the day of admission before therapy, the mean of MMSE was 21.6 (14–29), 1 week after therapy it was 22.7, and 2 weeks after therapy it was 24.3. The change at the end of 1 and 2 weeks were statistically significant ($P = 0.024$ and $O < 0.001$, respectively). At the day of admission before therapy, the mean of HAM was 9.9 (3–25), 1 week after therapy it was 7.8, and 2 weeks after therapy

it was five. The change at the end of 1 and 2 weeks were statistically significant ($P = 0.014$ and $O < 0.001$, respectively).
Conclusion: The cognitive and emotional disturbances could be seen among neurobrucellosis patients. These disorders improve by antibiotic therapy, without any anti-depressive or anti-psychotic therapy.

O145 A cluster of community-acquired bacteraemias in an intravenous drug-using population due to a novel clonal strain of methicillin-resistant *Staphylococcus aureus*

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Background: *Staphylococcus aureus* is a common cause of bacteraemia in intravenous drug users (IVDU), with about 40 admissions yearly to our hospital. Isolates from these patients are usually susceptible to methicillin (MSSA) but we noticed a recent increase in bacteraemias attributable to methicillin-resistant organisms (MRSA).
Objectives: To investigate the microbiology and epidemiology of a cluster of MRSA bacteraemias in the local IVDU population.
Methods: Sensitivity testing of *S. aureus* isolates was performed to BSAC recommendations. MRSA isolates were typed by pulsed-field gel electrophoresis (PFGE) and molecular work was carried out to determine the staphylococcal chromosomal cassette type (SCCmec) and presence of specific pathogenicity factors. A case note review was performed to ascertain epidemiological differences between IVDU with MRSA and those with MSSA.
Results: From January 2001 to June 2003, 21 patients had MRSA bacteraemia with a strain possessing a novel antibiogram (resistant to erythromycin and fusidic acid, susceptible to ciprofloxacin). The 'new strain' MRSA was clonal on PFGE and the SCCmec was typed as group 1. In 2001, 2002 and the first half of 2003 there were two, nine and 10 patients, respectively. Nineteen of 21 patients with 'new strain MRSA' were IVDU. During this period 90 other IVDU were admitted with MSSA bacteraemia. The mean age (32 years) was the same in both cohorts of IVDUs, but there were relatively more males (17/19) in the MRSA than in the MSSA cohort (73/91). The source of MRSA sepsis was often groin or lower limb infections and many of the patients were poly-drug misusers.
Conclusions: (i) MRSA bacteraemia in IVDU is a new phenomenon in Liverpool. (ii) The 'new strain MRSA' is clonal and distinct from nosocomial MRSA prevalent in our region. (iii) Initial review suggests no epidemiological differences between IVDU with MRSA and those with MSSA and this is being investigated further.

O146 Clinical and microbiological features of *Nocardia* infection during a period of 7 years

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Objectives: Recent application of modern taxonomic procedures, including molecular characterisation, has expanded our knowledge of the genus *Nocardia*. *Nocardia* species can vary in their antimicrobial susceptibility patterns. We present the identification and susceptibility pattern of *Nocardia* isolated in our hospital during a 7-year period and its correlation with clinical presentation and outcome.
Methods: Twenty-five patients with *Nocardia* infection were diagnosed in a University Hospital in Barcelona, Spain, from 1997 to 2003. Different *Nocardia* species were identified by 16S rRNA sequencing. Sensitivity to cotrimoxazol, imipenem, cefotaxime, amikacin and linezolid were determined by E-test. Age, comorbidity parameters, and spectrum of disease of *Nocardia* infection were collected. Outcome measures were: days of hospital stay, readmission, intensive care unit admission, crude and attributable mortality. Statistical analyses were performed with a Chi-square test (SPSS statistics package).
Results: The mean age was 69 years (SD = 12.63). Seventeen patients (68%) were male and 17 over 65 years. Charlson comorbidity index was 2.9 (range 1–7). Eighteen patients (72%) had chronic obstructive pulmonary disease (COPD). Seven patients (25%) had cellular immunosupression (HIV infection, chronic corticotherapy, immunosupressive drugs and haemathologic neoplasias). Twenty-three of the 25 patients (92%) had pulmonary infection, three skin involvement, and one cerebral nocardiosis. Nine isolates (36%) were *N. farcinica*, six (24%) *N. abscessus*, five (20%) *N. cyriacigeorgica*, two *N. otitidiscaviarum*, one *N. veterana*, one *N. nova* and one *N. asteroides sensu stricto*. Crude mortality was 56% (14 patients), and attributable mortality was 36% (nine patients). *Nocardia farcinica* had higher mortality (88.9%) than others, followed by *N. abscessus* (80%) ($P = 0.032$). All isolates were sensitive to cotrimoxazol, amikacin and linezolid.
Conclusions: *Nocardia* infection usually affects patients with active comorbidity, mainly COPD. There were no differences in comorbidity and clinical spectrum among species of *Nocardia*. *Nocardia farcinica* is the most common species found in our study. *N. farcinica* and *N. abscessus* were associated with higher case-fatality rates. Cotrimoxazol, amikacin and linezolid showed excellent sensitivity *in vitro*.

Resistance surveillance: Local hotspots, global strategies: a focus on common RTI pathogens (Symposium arranged by GlaxoSmithKline)

S155 Introduction: resistance, surveillance and beyond

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Antimicrobial resistance is an increasing problem worldwide. Understanding the changes in resistance over time, and how these changes might be linked to antimicrobial usage in particular countries is one of the critical elements in combating resistance. Similarly, understanding the underlying mechanisms by which pathogens express resistance is crucial. Surveillance data can highlight hotspots of resistance and can help to predict future growth in the prevalence of resistance by consideration of resistance data together with antibiotic usage and other relevant factors in specific geographical areas. Assessing the factors that lead to resistance, and the impact of resistance on the individual patient and the community is necessary if antimicrobial

resistance is to be adequately addressed. Additionally, comparing countries with a high prevalence of resistance with those that have a lower prevalence of resistance may contribute to understanding the factors driving resistance, and highlighting those efforts which help to prevent the development and spread of resistant organisms. Strategies to overcome resistance are needed if antimicrobials are to retain their usefulness in the clinical environment.

S156 Surveillance hotspots: the highs and lows of regional resistance. Russia

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Introduction: Resistance of microorganisms to antimicrobials is an inevitable and worldwide phenomenon. Despite the availability of reliable global surveillance data, it is very well understood that antimicrobial resistance has substantial geographical variations, thus dictating the need to perform regional surveillance studies. Striking differences are becoming evident in Europe, with countries such as Spain and France reporting extremely high penicillin-, erythromycin- and multi-drug resistance in *Streptococcus pneumoniae*, and others (UK, Germany, Russia) traditionally being considered areas with low prevalence. In the following presentations, an update on the resistance of respiratory tract pathogens in various regions will be reviewed in detail, with a special focus on *S. pneumoniae* and the clinical implications of these data.

Russia: This presentation provides an update on the current state of antimicrobial resistance of community-acquired pathogens in Russia based on the results of multicentre studies, organised under the auspices of the Institute of Antimicrobial Chemotherapy (IAC) and the Center for Monitoring of Antimicrobial Resistance of Ministry of Health of Russia (CMARR). NCCLS methodology was used for all studies. Among 581 clinical strains of *S. pneumoniae* isolated in 2001–2003 from 25 centres, 10.2% were nonsusceptible to penicillin (with only 1.9% with high-level resistance) and none were found to be nonsusceptible to aminopenicillins (amoxicillin, amoxicillin/clavulanate) or third-generation cephalosporins. With regard to macrolides, 8.6–9.0% of strains were resistant to different 14- or 15-membered macrolides while 16-membered representatives (midecamycin) and lincosamides (clindamycin) were more active (4.0 and 3.3% resistance, respectively). However, regional analysis indicated that nonsusceptibility to erythromycin, azithromycin, midecamycin and clindamycin was significantly higher ($P < 0.05$) in Central Russia compared with Siberia and Ural. The highest nonsusceptibility rate detected was against tetracycline and co-trimoxazole (27.5 and 31.5%, respectively) with no geographical variations. There were 2.1% of strains with decreased susceptibility to ciprofloxacin (MIC > 4 mg/L). No resistance was found to respiratory fluoroquinolones (levofloxacin and moxifloxacin). A recent study of nasopharyngeal pneumococci in 4135 children under 7 years old from 91 organised communities in 19 cities of European and Asian Russia showed the higher resistance in such strains on comparison with clinical isolates with orphanages being 'hotspots' of resistance. These data suggest that residential institutions, namely orphanages, could be reservoirs for the development and further spread of resistant isolates. This hypothesis should be confirmed in further studies, potentially involving long-term care facilities. Ampicillin resistance among *Haemophilus influenzae* was 4.9%, with no resistance to amoxicillin/clavulanate (PeHA-Sus-I, phase 'A' study). *Moraxella catarrhalis* seems to be of low epidemiological importance in Russia, being isolated only in rare cases from patients with otitis media and acute exacerbations of chronic bronchitis, with all strains being β-lactamase producers and retaining susceptibility to β-lactam/β-lactamase-inhibitors.

S157 Surveillance hotspots: the highs and lows of regional resistance. Central and Eastern Europe

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Antimicrobial resistance is recognised as a global problem, with certain geographic regions particularly affected. Recent surveillance studies have reported the regional prevalence of penicillin-resistant *Streptococcus pneumoniae* (PRSP, penicillin MICs $\geq$2 mg/L) to be between 7.8 and 16.6%, depending on the study cited, for the Central and Eastern European region. However, these values can vary considerably between countries, from as low as 1.8% in the Czech Republic (Alexander Project, 1998–2000) to 37.0% in the Slovak Republic. Empiric prescribing is further complicated by the fact that many PRSP isolates are also resistant to other classes of drugs, particularly macrolides. Macrolide resistance has been reported in >35% of *S. pneumoniae* isolates from some Central and Eastern European countries, and as many as 34.5% of PRSP identified in the Alexander Project in Central and Eastern Europe were also resistant to erythromycin. Quinolone resistance among *S. pneumoniae* remains rare, but has begun to emerge in this region, particularly in Croatia and Hungary. β-Lactamase production is the primary mechanism of resistance in the respiratory pathogens *Haemophilus influenzae* and *Moraxella catarrhalis*. Among *H. influenzae* from Central and Eastern Europe in the Alexander Project, the prevalence of β-lactamase-positive isolates ranged from 5.5 to 8.3%. Among *M. catarrhalis*, 91.5% of isolates from this region were β-lactamase producers. Resistance among these common respiratory pathogens continues to be of concern, but countries with a low prevalence of resistance provide opportunities to examine the differences between these countries and those with a higher prevalence of resistance to evaluate the factors driving resistance. Continued surveillance is needed to monitor the activity of available antimicrobials, and those in development, in order to help guide effective prescribing.

S158 Surveillance hot spots: the highs and lows of regional resistance. Europe North and South

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Barcelona, E

Antimicrobial resistance among respiratory pathogens, including *Streptococcus pneumoniae* (SP), can vary greatly between regions and countries. In the Alexander Project (2001), the regional prevalence of penicillin-resistant SP (PRSP, penicillin MICs $\geq$2 mg/L) in Southern Europe (France, Greece, Italy, Portugal and Spain) was 21.0%, ranging between 4.9% (Italy) and 46.0% (France), and of macrolide-resistant SP (erythromycin MICs $\geq$1 mg/L) was 29.8%, ranging from 8.0% (Portugal) to 56.4% (France). PRSP prevalence in Northern Europe (Belgium, Germany, the Netherlands, Switzerland and the UK) was between 1.1% (UK) and 9.4% (Switzerland), and was 5.5% overall. The regional prevalence of macrolide-resistant SP was 17.7%, ranging from 7.5% (Germany) to 33.2% (Belgium). High rates of penicillin-macrolide co-resistance were also reported in both Northern and Southern Europe, with 67.7% and 62.6% of PRSP in these regions, respectively, resistant to erythromycin. The PROTEKT study (2002) showed similar overall rates of PRSP and macrolide-resistant SP in these regions. In Southern Europe, the PRSP prevalence ranged from 5.6% (Italy) to 47.7% (France), and the prevalence of macrolide-resistant SP from 12.9% (Portugal) to 60.7% (France), while in Northern Europe, PRSP prevalence was between 0 (Netherlands) and 8.7% (Switzerland), and macrolide-resistant SP between 11.9 (Netherlands) and 32.1% (Belgium). The PRSP prevalence reported by the EARSS network (2002, invasive isolates only) was generally lower than that seen in the other studies. Macrolide nonsusceptibility among SP isolates collected by EARSS ranged from 0 (Portugal) to 58.0% (France). The prevalence of PRSP in Scandinavian countries was low (all studies), but in recent years the prevalence of macrolide-nonsusceptible SP approximately doubled in Finland [EARSS:

6.0% (1999) to 14.0% (2002)] and Sweden [PROTEKT: 4.7% (2000)–9.3% (2002)]. The prevalence of β-lactamase-positive *Haemophilus influenzae* was greater in Southern than in Northern Europe, and >90% of *Moraxella catarrhalis* isolates were β-lactamase-positive (all studies). The differences in the prevalence of resistant strains of SP identified in the countries of northern and southern Europe indicate that factors driving resistance, such as prescribing practices and infection control, also vary considerably in these countries. The rapid increase in the prevalence of macrolide-resistant SP in at least two Scandinavian countries is of concern.

S159 Surveillance hotspots: the highs and lows of regional resistance. USA and global

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Antimicrobial resistance among respiratory pathogens, particularly *Streptococcus pneumoniae*, is now prevalent in many parts of the world, including the USA. One-quarter to one-third of *S. pneumoniae* isolated in surveillance studies in the USA in 2001–2002 were penicillin resistant (penicillin MICs $\geq$ 2 mg/L). Globally, around 20% of *S. pneumoniae* isolates collected in 2001–2002 were penicillin resistant. Regional variations in the prevalence of penicillin-resistant *S. pneumoniae* exist both within the USA and globally. Within the USA, the prevalence of penicillin-resistant *S. pneumoniae* ranged from 14 (northwest region) to 30% (south-central region) in the Alexander Project in 2001. Globally, regions such as the Far East and Central/Eastern Europe are known to have particularly high rates of penicillin resistance among *S. pneumoniae* isolates. Macrolide resistance (erythromycin MICs $\geq$ 1 mg/L) now exceeds penicillin resistance among isolates of *S. pneumoniae* in the USA and globally. In 2001, for example, the Alexander Project reported the prevalence of macrolide-resistant *S. pneumoniae* to be 28% in the USA and 30% worldwide. Of great concern as well is that $\geq$75% of penicillin-resistant *S. pneumoniae* are also resistant to macrolides, both in the USA and worldwide. Although quinolone-resistant *S. pneumoniae* have been reported, mainly in Hong Kong, Canada, Spain, Hungary and Croatia, the global prevalence of these strains remains <2%. Among *Haemophilus influenzae*, β-lactamase production has been identified in one-quarter of isolates from the USA in 2001. Globally, the prevalence of β-lactamase-positive *H. influenzae* is lower – around 15% in 2001–2002. Empirical prescribing is further complicated in infections where *H. influenzae* is common, as virtually all strains of this organism are intrinsically resistant to macrolides and azalides via an efflux mechanism; this presents an additional problem as most isolates of *H. influenzae* are categorized as susceptible by NCCLS criteria. Quinolone resistance in *H. influenzae* has been described, but is currently very rare (<0.5%). More than 90.0% of *Moraxella catarrhalis* isolates worldwide are β-lactamase-positive. These levels of resistance, and the high prevalence of multiply resistant organisms, demonstrate that new antimicrobials active against resistant strains are needed. An increased awareness of resistance and strategies to prevent its further development and spread are needed in the USA and on a global scale.

S160 Factors behind the resistance

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Barcelona, E

The prevalence of resistance to common antimicrobials among respiratory pathogens continues to increase and is beginning to emerge in previously unaffected areas. The differences seen between regions in the prevalence of resistance suggest that different factors are at work in different areas to drive resistance. This is exemplified by surveillance data from across European regions. Known factors in the development of resistance include inappropriate choice or inadequate dosing of antibacterials, unnecessary prescribing, and poor patient compliance, along with the spread of resistant clones. Macrolide resistance among *Streptococcus pneumoniae* is a growing concern, as this now exceeds penicillin resistance in many areas. The introduction of long-acting macrolides may be a factor driving this increase in resistance and needs further examination. Quinolone resistance, although infrequent, represents a considerable clinical challenge, as these agents are often reserved for patients with more severe disease or those who have failed previous antimicrobial therapy. The primary resistance mechanism in *Haemophilus influenzae* is β-lactamase production, which inactivates unprotected β-lactam antibacterials. Recent research has demonstrated that most strains of *H. influenzae* also have an innate macrolide-efflux mechanism. There is an increasing body of data showing the clinical relevance and consequences of these resistances. Resistance is no longer a local or homogenous phenomenon, such that within a region 'hotspots' of resistance can occur, and this is particularly evident across Europe. Areas of low resistance also exist, and these, when evaluated with data on prescribing practices and other factors, can provide information on effective resistance control. An awareness of resistance patterns, and how they are changing over time, together with appropriate prescribing and infection control methods are needed to address antimicrobial resistance. Novel antimicrobials and new formulations of existing agents, capable of eradicating resistant pathogens, also have a key role to play in the containment of resistance.

S161 Surveillance for the future: global strategies to control resistance

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The prevalence of antimicrobial resistance among common respiratory pathogens continues to increase. Surveillance is a key component in the effort to document this and provide information on which agents have the best activity. To be of most value, surveillance needs to be able to provide up-to-date information on local susceptibility patterns. National and international surveillance is also critical for monitoring the ongoing development and spread of resistance, and to help predict its future spread. Ongoing surveillance in regions where resistance does not currently occur will allow a better understanding of the factors associated with the emergence of resistance when it develops. While local clinical laboratories can provide surveillance data on a local level, more comprehensive surveillance studies require greater resources and standardised methods of collection, analysis and quality control. Alone, surveillance describes the current state of resistance, but should be combined with other data, such as prescribing statistics and patient demographics, to evaluate associations between resistance, drug prescribing and disease risk factors. Academic institutions, government organisations and the pharmaceutical industry can all contribute in this area. Comparisons of resistance data for different geographical regions, and analysis of those data in light of resistance-driving factors can provide information towards establishing optimal practice guidelines. Susceptibility data should be interpreted according to pharmacokinetic/pharmacodynamic parameters, as many interpretative breakpoints in current use in the USA are not valid, particularly for *H. influenzae*. Global data also provide a basis for phenotypic analysis and tracking of genotypic clones. Long-term storage of isolates allows retesting against new antimicrobials in development to assess their potential clinical utility. Surveillance data can help guide prescribing by indicating areas in which certain antimicrobials are at risk of losing their clinical efficacy by revealing the most commonly isolated pathogens in that region and their susceptibility patterns. By increasing physician awareness about the prevalence and spread of resistance, surveillance can reinforce the message of the need for appropriate prescribing. Resistance is inevitable in this era of antimicrobial use, and surveillance will continue to play an important role in monitoring its development and adapting to these findings.

Catastrophes after crossing species barriers: lessons from SARS, influenza…

S162 Catastrophes after crossing species barriers: lessons from SARS, influenza…

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Probably the most tragic examples of virus infections that have caused the deaths of many millions of people in the past century were the influenza and AIDS pandemics. These events occurred as a direct result of the introduction of animal viruses into the human population. Similarly, mass mortalities among aquatic and terrestrial mammals were caused by the introduction of viruses into species in which they had not previously been present. It seems paradoxical that at a time when we have managed to control or even eradicate major human virus infections like polio and smallpox we are increasingly confronted with new or newly emerging virus infections of humans and animals. A complex mix of social, technological and ecological changes, and the ability of certain viruses to adapt rapidly to a changing environment, seems to be at the basis of this phenomenon. Extensive diagnostic and surveillance networks, and novel vaccine- and antiviral development strategies should provide us with the safeguards to limit its impact.

Global pandemics: how well is the global village prepared to deal with emerging infectious diseases

K170 WHO global response to SARS

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Background: On 15 March 2003, the World Health Organization (WHO) issued an emergency alert to a severe respiratory disease, of undetermined cause, that was rapidly spreading along the routes of international air travel. The coordinated global response that followed marked the first time that information about a newly emerging disease was gathered, in real time, by electronically interlinked networks of experts, and made available to the world at large as the outbreak unfolded.
Method: The response to SARS relied on new mechanisms for detecting unusual disease events and limiting their harm. To expedite outbreak detection, the Global Public Health Intelligence Network (GPHIN), developed and maintained by Health Canada, was set up for the real-time systematic gathering of disease intelligence while WHO made use of its unique and long-standing international experience in infectious diseases control to support global alert and response operations. The Global Outbreak Alert and Response Network (GOARN), currently uniting 120 partners, was established as a 'strike force' of specialized staff and technical expertise on stand by for emergency investigations and on-the-spot assistance. A system of electronic communications was extended to all 141 WHO country offices. The network of collaborating centres was expanded. New procedures for outbreak verification and standardized protocols for all phases of outbreak response were developed. SARS tested the simultaneous performance of these mechanisms under emergency conditions.
Result: The response to SARS tested an assumption of fundamental importance to public health: all these safeguards, working at their best, might be able to prevent a new disease from establishing endemicity, and thus spare the world untold suffering and expense. From the outset, this was the goal pursued by WHO in coordinating the activities of many partners.
Conclusion: The containment of SARS within 4 months following its recognition as an international threat provides some reassurance that international partnership taking advantage of the WHO global framework makes the world better prepared to defend itself against new diseases.

The changing faces of *S. aureus*

S173 The worldwide emergence of hyper-virulent MRSA in the community

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Lyon, F

Methicillin-resistant *Staphylococcus aureus* (MRSA) strains have been considered during two to three decades as the typical bacterial population present in hospital settings and as a major cause of hospital-acquired (HA) infections. Community-acquired MRSA (CA-MRSA) emerged worldwide at the end of the last century. There is a confusion in the literature between healthcare related MRSA infections which occurred in patients with a past history of hospitalization and are due HA-MRSA strains, and true CA-MRSA infections due to strains that are not present in the hospital settings. Demographic characteristics of HA-MRSA infections differed from those of CA-MRSA, the former occurring mainly in elderly whereas the later occurring in young people. HA-MRSA infections are facilitated by the presence of skin effraction after surgery or intravenous indwelling catheter, whereas CA-MRSA infections are mainly primary-skin infections occurring in patients with no initial skin effraction. The Panton–Valentine leucocidin present in CA-MRSA strains all over the world represents, by its necrotic activity, one of the virulence factors possibly associated with cutaneous tissue destruction. These PVL-positive CA-MRSA strains are easily transmissible within families, but also on a larger scale, in communities with high level of promiscuity (in prisons, schools, sport teams, etc.). The simple skin to skin contact seems to represent the way of transmission. The skin infection often appears initially as an insect bite. The same clone of CA-MRSA strains have been detected in several European countries demonstrating its extended spread in the community. It has invaded France, Switzerland, Belgium, Germany but also the Nordic European countries. Several different CA-MRSA clones are spreading in US and in Australia and New Zealand. Despite a different genetic background, a frequently common trait of these clones is the presence of the PVL genes and the small-sized SCCmec type IV cassette. More recently other CA-MRSA clones

containing other virulence genes such as those encoding the exfoliatin or the toxic shock toxin are also emerging.

In some area of the world, CA-MRSA already account for >70% of the *S. aureus* isolated in the community, showing that *S. aureus* strains could progressively all become resistant to methicillin. The ultimate issue is to set up and implement the adequate prevention measures to reduce or limit the spreading of these strains.

S174 MRSA incidence and control in European hospitals

P. Urbášková
Prague, CZ

Infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) have been reported in European hospitals for more than 40 years, with increasing frequency in the last decade. Multiple factors have been involved in the emergence of MRSA, one of the most important being their capability of adapting to hostile environments and of spreading rapidly. MRSA have currently been the worldwide major cause of hospital-acquired infections that are difficult to treat and control because of emerging resistance to almost all antimicrobial agents. The recently published reports on the incidence of hospital infections caused by vancomycin-resistant MRSA and community infections caused by MRSA in individuals without risk factors give evidence for changes in epidemiology of MRSA.

Antimicrobial resistance in European countries has been monitored since 1999 within a vast internationally accepted initiative EARSS (European Surveillance Antimicrobial Resistance System). Analysis of comparable and validated data from more than 1000 hospitals of 28 European countries revealed substantial and permanent differences in the MRSA prevalence among countries and hospitals. This can be explained by differences in antibiotic consumption, antibiotic prescription patterns and implementation of and compliance with infection-control measures. The identification of localities with endemic long-term high incidence of MRSA would be of relevance for analysis of the mechanisms involved in transmission of resistant clones, their persistence in the population and resistance development. The findings would be used as background information for implementation of an effective strategy for reduction and prevention of MRSA infections in Europe.

S175 Small colony variants of *S. aureus* – clinical significance and detection challenges

B. Kahl
Munster, D

SCVs represent a subpopulation of *S. aureus* with small, nonpigmented, nonhaemolytic colonies on Columbia blood agar plates compared with the growth of normal *S. aureus*. SCVs of *S. aureus* have been recognised since decades. Over the past 7 years, a clinical syndrome has been defined as typified by persistent, recurrent and antibiotic-resistant staphylococcal infections. SCVs have been isolated from diseases such as osteomyelitis, arthritis, abscesses and from airway secretions of CF patients. SCVs produce greatly reduced amounts of alpha-toxin, thereby allowing SCVs to persist intracellular in *in vitro* systems. Most clinical SCVs were impaired in their electron transport or thymidine synthesis. Due to the unusual colony morphology, slow growth and possible atypical appearance in Gram staining, *S. aureus* SCVs are easily missed or misdiagnosed in the routine laboratory resulting in major reporting errors with regard to their presence and susceptibility.

Diarrhoeal diseases (Joint symposium arranged with the IDSA)

S177 Shiga toxin-producing *Escherichia coli*

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Shiga toxin (Stx)-producing *Escherichia coli* (STEC) cause a broad spectrum of diseases in humans ranging from mild diarrhoea through haemorrhagic colitis to haemolytic uraemic syndrome (HUS). The most important STEC serotype implicated worldwide is O157:H7. However, several so called non-O157 STEC serotypes have emerged. After a mean incubation period of 3 days, patients develop watery diarrhoea accompanied with cramping abdominal pain. During next days, bloody diarrhoea may occur. One week after the onset of diarrhea, in about 15% of patients under 10 years STEC infection results in a systemic complication, the HUS. Long-term sequelae such as proteinuria, hypertension, reduced renal function, or neurological residuals occur in about one-third of the patients who recover from HUS. Stx are considered the major virulence factors of STEC involved in the pathogenesis of this extraintestinal manifestation. The toxins cross the intestinal barrier and bind to endothelial cells of the target organs. They injure the host cells by inhibition of protein synthesis, stimulation of prothrombotic messages or induction of apoptosis. HUS is a net effect of a variety of interacting factors, including host factors (such as age), exogeneous factors (infectious dose, administration of antibiotics) and virulence characteristics (Stx type, the presence of intimin, serine proteases) of the infecting STEC strain. All known STEC virulence determinants are located on mobile genetic elements and this has an important impact on the evolution of these pathogens. Domestic animals, especially cattle, are the major reservoirs of STEC. The principal ways of STEC transmission include contaminated food, contaminated water, person to person transmission and direct contact with animals. Most of STEC strains cannot be identified using conventional culture procedures. Detection of stx genes and/or Stx using polymerase chain reaction and enzyme-immunoassay represents an effective, serotype independent method for screening of STEC. The recent progress in understanding the pathogenesis and epidemiology of STEC infections forms a basis for the development of future strategies to prevent STEC infections in humans.

New technologies for microbiological diagnostics (Joint symposium arranged with ICAAC)

S183 Advances in the microbiologic diagnosis of biofilm-associated infections

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Microorganisms attach to surfaces and form biofilms. Biofilm formation has been implicated in the pathogenesis of a wide range of human infections, especially those associated with devices such as orthopaedic hardware, pacemakers, prosthetic heart valves, and intravascular catheters. Biofilm cells are associated with an extracellular polymeric substance matrix, and exhibit reduced growth rates, in the context of altered-regulation and/or the presence of specific genes. Most biofilm studies have focussed on the underlying pathophysiology of biofilm formation and the implications of the biofilm phenotype on therapeutics. In contrast, little

attention has been directed towards the microbiological diagnosis of device-related biofilm-associated infections.

Topics to be explored during this session include new approaches to the removal of bacteria from medical devices for the purpose of diagnosing infection and the use of genetic markers of biofilm formation for the diagnosis of biofilm-associated infections.

Molecular epidemiology and management of nosocomial outbreaks

O184 Czech epidemic strain of *Burkholderia cenocepacia* does not belong to ET12 lineage

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Objectives: Patient-to-patient transmissible strains of *Burkholderia cepacia* complex account for the infection spread among patients with cystic fibrosis (CF). Epidemic outbreaks in Canada and the UK were caused by highly virulent ET12 clone that is characterized by cbIA-encoded cable pili. In the Czech CF population, an epidemic strain within *B. cenocepacia* (former genomovar III of *B. cepacia* complex) had been also identified. Although molecular typing data indicated sufficient genetic distinction between the Czech epidemic strain and ET12, the only tool to prove the ET12 occurrence or absence in the Czech CF population is based on the detection of cbIA gene.
Methods: We examined 81 isolates from 65 Czech CF patients who had been previously demonstrated to share the same epidemic strain using RAPD and PFGE typing. All the isolates were subjected to PCR detecting cbIA gene. Simultaneously, we used PCR targeting 16S rDNA region as an internal control of successful amplification. PCR products were visualised on the 2% agarose gel stained with ethidium bromide.
Results: All 81 examined isolates were cbIA-negative, while a positive signal was seen in ET12 reference strains used as a positive control.
Conclusions: The epidemic *B. cenocepacia* strain present in the Czech CF population does not possess the nucleotide sequence encoding cable pili unique for ET12 strain. Thus, the Czech CF population is not confronted with highly virulent variant of transmissible strains, i.e. ET12. To find out the origin of the *B. cenocepacia* infection epidemic in the Czech CF patients, comparisons with other previously described transmissible strains abroad are being performed. Supported by Ministry of Health (grant 6568-3) and Ministry of Education (grant 111300003), Czech Rep.

O185 Genetic diversity of penicillin-nonsusceptible clinical strains of *Streptococcus pneumoniae* isolated in Russia: results of a nationwide study

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Objectives: Antibiotic resistance in respiratory pathogens such as *Streptococcus pneumoniae*, is a global issue in many countries, including Russia. The purpose of the current study was to characterise the genetic diversity of penicillin-nonsusceptible *S. pneumoniae* (PNS SP) isolated from children and adult patients in different regions of Russia.
Methods: Seventy-four (17 from PeHASus-I, phase 'A' and 57 from PeHASus-I, phase 'B') penicillin-nonsusceptible SP isolates (MIC range 0.12–4 mg/L) obtained from patients of 1 month to 87 years in 19 Russian hospitals situated in 16 cities (Novosibirsk, Ufa, Irkutsk, Tomsk, Yaroslavl, Kazan, Krasnodar, Moscow, Stavropol, Ekaterinburg, Yakutsk, Ryazan, Tyumen, Novokuznetsk, St Petersburg, Kovrov) located in the areas of Western, Southern, Central Russia, Siberia, Ural and Far East during 2001–2002 underwent molecular typing by BOX-PCR with primer BOX-A (ATACTCTTCGAAAATCTCTTCAAAC) and RAPD-PCR with primer M13 (GAGGGTGGCGGTTCT). Cluster analysis of genetic fingerprints was performed by UPGMA algorithm with Pearson coefficient using the GelCompar software (AppliedMaths).
Results: The combined analysis of BOX- and RAPD-PCR patterns revealed 61 unique genetic types of which 10 clonal groups comprised from two to four isolates each, and the other 52 included single isolates. Clonal dissemination of PNS SP was mainly observed in the hospitals of Moscow and Novosibirsk. Thus, nine isolates from the Moscow City Clinical Hospital no. 23 produced very similar fingerprints differing from each other by three bands at most. Single case of clonal relatedness between the isolates from geographically distinct centers (Moscow and Novokuznetsk) was also found. However, the multiplicity of genetic types indicated that resistance to penicillin was acquired by numerous strains.
Conclusions: Combination of BOX- and RAPD-PCR is a useful tool for monitoring of circulating pneumococcal population. PNS SP isolated from Russian hospitals mostly represent genetically diverse population of strains. The same clone was found to be spread between faraway situated cities (Moscow and Novokuznetsk).

O186 Relationships between virulence factors of methicillin-resistant *Staphylococcus aureus* and clonal types

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Objectives: *Staphylococcus aureus* is associated with an increasing number of hospital infections, including endocarditis, deep-seated abscesses, bacteraemias and toxemic syndromes, as toxic shock syndrome (TSS). Methicillin-resistant *S. aureus* (MRSA) is a major nosocomial pathogen found throughout the world and in the University Hospital of Patras. We have investigated the presence of genes encoding the toxic shock syndrome toxin (TSST-1) and the Panton–Valentine leucocidin (PVL) among MRSA in relation to clonal types.
Methods: The MIC of oxacillin was determined by the agar dilution method in Mueller–Hinton agar supplemented with 2% NaCl, according to the guidelines of NCCLS. PBP2a production was investigated by a Latex agglutination test (bioMerieux) in all *S. aureus* with MIC > 0.5 mg/L. PCR amplification with specific primers was used for the detection of mecA gene and thus 60 MRSA collected from different patients were characterised during 2002. The lukS and lukF (encoding PVL components S and F) and tst (which encodes the TSST-1) genes were detected by PCR. Clonal types were determined by PFGE of chromosomal DNA SmaI digests.
Results: Luk gene was detected 36 MRSA (60%), mainly from severe skin infections. It was present in 100% of abscesses strains, 82% of skin lesions strains and 50% of wound infections strains. Tst gene appeared in eight strains (13%) from wound infections (5), skin lesions (1), urinary tract infection (1) and bacteraemias (1). PFGE classified the 60 MRSA into three clonal types. Type A included 11 strains (18%), type B 13 strains (22) and 36 isolates (60%) belonged to type C. All the MRSA of type C carried the luk gene, which was not detected in any strain of the other two types. The tst gene was detected in eight (73%) strains of type A only. Strains of clonal type B did not carry the genes tested.
Conclusions: The presence of luk gene in all PFGE type C strains is indicative of clonal dissemination, associated with necrotic lesions among hospitalized and outpatients. The tst gene is to a

less extent associated with another MRSA clone independently of the underlying disease.

O187 DNA fingerprinting of mycobacteria using automated repetitive sequenced-based PCR

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Objective: DNA fingerprinting of mycobacterial pathogens, including *Mycobacterium tuberculosis* (Mtb), *Mycobacterium avium* complex (MAC), and nontuberculous mycobacteria (NTM), has numerous applications in clinical microbiology and molecular epidemiology. Current genotypic methods used to type Mtb, MAC and NTM strains include RFLP, PFGE, Spoligotyping, and MIRU-based typing. Additionally, identification of mycobacteria other than tuberculosis (MOTT) is challenging and may include sequencing or traditional biochemical testing. These methods are either time consuming, laborious, expensive, or highly restricted with regard to applicability. This study reports the use of a simple, rapid and cost-effective rep-PCR technology for typing isolates of most mycobacterial species using the DiversiLab System.
Method: Fingerprints from 25 different ATCC *Mycobacterium* species were stored in a library. Twenty-five clinical isolates were gathered from two geographical regions and were grown on Lowenstein–Jensen media. DNA from each culture was extracted using the UltraClean™ Microbial DNA Isolation Kit. Seventy-five nanograms of each sample DNA was used to generate rep-PCR-based fingerprints using the DiversiLab *Mycobacterium* Kit, microfluidic chips, and the Agilent 2100 analyser. Sample analyses and data archiving were carried out using the DiversiLab System.
Result: Dendrogram and gel-like images of rep-PCR profiles for all 25 different ATCC *Mycobacterium* species and all MOTT clinical isolates were obtained using DiversiLab System. The rep-PCR clustering identified MOTT strains and showed excellent concordance with similar ATCC species and biochemical characterization. Similarly, different isolates of rapidly growing mycobacteria and slow-growing NTM were fingerprinted using the DiversiLab *Mycobacterium* Kit.
Conclusion: Rep-PCR has distinctive advantages for mycobacterial typing such as ease of use, requirement of relatively small amounts of DNA, the potential for typing strains not typeable by RFLP due to low IS6110 copy number, and the ability to type other mycobacterial species. Based on this pilot study, the DiversiLab System shows considerable promise as a tool for identification and typing of MOTT.

O188 Molecular epidemiological analysis of *Mycobacterium tuberculosis* isolates reported in England in 1998

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Background: The resurgence of tuberculosis (TB) since the mid-1980s has revised public health concern in the UK.
Design: All culture-confirmed cases of TB reported in England from 1st January to 31st December 1998 were identified. All isolates were typed using RFLP. spoligotyping was used as a secondary typing method for low copy number (LCN) isolates. Epidemiological information was gathered from the UK Mycobacterial Resistance Network (Mycobnet) and the 1998 National TB survey in England and Wales. Additional information was collected for 'clustered' cases and a set of control cases to assess risk factors for clustering.
Objectives: (i) to analyse the distribution of MTB in England and Wales in 1998; (ii) to examine the occurrence of clusters of indistinguishable isolates and to identify risk factors associated with clustering.
Results: A total of 3396 culture confirmed cases of MTB were identified of which 2265 produced IS6110 RFLP fingerprints; 1808 (80%) isolates were high copy number (HCN), i.e. those with five or more copies of IS6110 and 457 (20%) were LCN. The majority of strains (64%) had between seven and 14 bands. There were 152 HCN clusters (two or more isolates) comprising 372 isolates. The degree of recent transmission in cases with HCN fingerprints was determined to be 12%. LCN isolates were spoligotyped producing 42 LCN clusters made up of 213 isolates. Analysis of the HCN isolates showed the following risk factors for clustering: having known previous treatment (OR 3.85, 95% CI 2.21–6.71), having pulmonary or respiratory disease (OR 1.70, 95% CI 1.16–2.48) and showing resistance to at least one antimicrobial drug (OR 1.71, 95% CI 1.15–2.54). In a case-control study, homelessness was shown to be a risk factor for clustering (OR 5.57, 95% CI 1.26–24.7).
Conclusions: Molecular techniques have greatly enhanced our understanding of the transmission dynamics of TB disease. Combined with epidemiological methods they provide a tool for an efficient surveillance system for the control of tuberculosis.

O189 Use of statistical process control for early detection of hospital-acquired outbreak of alarm bacteria

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Rud, N

Objective: Plotting cumulative retrospective data (c-chart) is one method of registering nosocomial infection outbreaks. However, plotting data in statistical process control (g-chart) increases sensitivity significantly of outbreak identification compared with c-chart.
Methods: Laboratory data include information about nosocomial infections. In our examples we included unique data for *P. aeruginosa* and *Pseudomonas* sp. from Rikshospitalet and Sykehuset Asker og Bærum (SAB) and *S. aureus* data from SAB. The data were plotted retrospectively (c chart, quarterly observations) and by means of statistical process control (SPC) in g chart (rate, i.e. 'number of days between new patients with *S. aureus* or *P. aeruginosa*').
Results: Quarterly *Pseudomonas* numbers (c chart) increased significantly for both hospitals in the first quarter of 2002 compared with previous quarters. This increase was because of a national outbreak of *Pseudomonas* associated with a commercial mouth swab. The identification of this *Pseudomonas* outbreak was delayed when data was plotted in c chart. By plotting data as rate vs. observation number (g chart), analysis by SPC would have identified this national *Pseudomonas* outbreak in SAB about 6 weeks earlier ($P < 0.05$) than the warning given to Norwegian hospitals by the National Institute of Health. As a consequence hereof, *S. aureus* and *Pseudomonas* data for SAB are now plotted routinely in Excel as rate of new patients with these bacteria vs. observation number and compared with average rate (last 30 observations). On a given observation below average rate, information is given to the ward as well as the laboratory. This routine has made it possible to (i) alarm wards early of possible outbreaks and (ii) keep isolates for later fingerprinting analysis.
Conclusion: Our two examples show that statistical process control is a valuable tool for early identification of outbreaks with hospital acquired bacteria like *S. aureus* and *Pseudomonas*. Future hospital infection control will benefit significantly by use of SPC.

O190 Failure of classical surveillance strategy to detect a large hospital-wide outbreak of a multidrug-resistant *Enterobacter cloacae*

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Objectives: To identify the determinants causing the failure of a classical surveillance strategy (CSS) to detect a large outbreak of a

multidrug resistant (MDR) *Enterobacter cloacae* (EC). CSS refers to a strategy based on the recognition of an increased incidence of a species with a particular antibiogram at certain wards in a limited time period.
Methods: A hospital-wide increase in the number of MDR clinical EC isolates was detected in December 2002. Records were reviewed for the period from January 2001 to Dec 2002 (period I) to identify patients with cultures positive for EC during their stay in the hospital. From January to September 2003 (period II) EC isolates were prospectively stored. Genotyping by PFGE was done on all available EC isolates.
Results: A total of 466 patients were identified for being EC culture positive. From period I, 105 tobramycin-resistant (TR)EC (60 patients) and 27 tobramycin susceptible (TS)EC (24 patients), and from period II, 58 TREC (48 patients) and 49 TSEC (47 patients) were available. PFGE showed that 53 patients in period I and 34 patients in period II carried a TREC that belonged to one clone that was subject to evolution. The susceptibility patterns for eight non-beta-lactam antimicrobials were analysed for all 239 genotyped isolates. The clonal strain expressed 37 different antibiograms (including 2 tobramycin S variants) of which 1/4 were shared by ECs of other genotypes. The clonal strain was isolated from a large variety of clinical sites, including 30% normally sterile sites (eg BC, CSF). During period I cases were detected on nine different divisions (three ICUs and 18 wards). Patients were frequently transferred between wards (median 3) and divisions (median 2) and long laps in time were seen between cases (mean 2 weeks; range 0–18).
Conclusions: The determinants causing the failure of a CSS to detect this outbreak were: (1) the low incidence, (2) the long time interval between cases, (3) the hospital-wide occurrence of new cases, (4) the large variety of clinical sites from which the clone was isolated, and (5) the high variability of the antibiogram. These results illustrate the limitations of the CSS and stress the need of molecular typing facilities and (data mining) surveillance systems that integrate laboratory and hospital information systems to identify patterns indicative for the occurrence of hospital infections.

O191 Investigation of the association between the MRSA colonisation pressure and the occurrence of nosocomial MRSA infections in 75 German intensive care units

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Objectives: To investigate the association between the MRSA colonisation pressure and the nosocomial MRSA infection rate in 75 German intensive care units (ICUs).
Methods: 75 ICUs participating in the German Nosocomial Infection Surveillance System (KISS) recorded not only the number of nosocomial infections and patient days; they also determined the number of all MRSA cases representing the MRSA colonization pressure in the individual ICUs (i.e. the number of colonized as well as infected patients, nosocomial cases as well as patients with MRSA on admission to the ICU) for the first half year of 2003. Thus it was possible to associate the MRSA case rate with the nosocomial MRSA infection rate for the individual ICU. The median line of the nosocomial MRSA infection rate and the MRSA case rate divide the ICUs into four quadrants and differentiate between ICUs regarding the spread of MRSA.
Results: A total of 398 MRSA cases and 120 854 patient days were observed during the study period. 229 MRSA cases were already present upon patient admission to the ICU, the remaining 169 cases were considered nosocomial (i.e. not known at the time of admission nor detected during the first 48 h in the ICU). In all, 95 nosocomial infections were identified (= 24% of all MRSA cases). The median MRSA cases rate was 2.3, the 75th percentile 4.0 per 1000 patient days. The median nosocomial MRSA infection rate was 0.6 per 1000 patient days, its 75th percentile being 1.1 per 1000 patient days. The association between the MRSA case rate and the nosocomial MRSA infection rate for each of the 75 ICUs is shown in Figure 1.

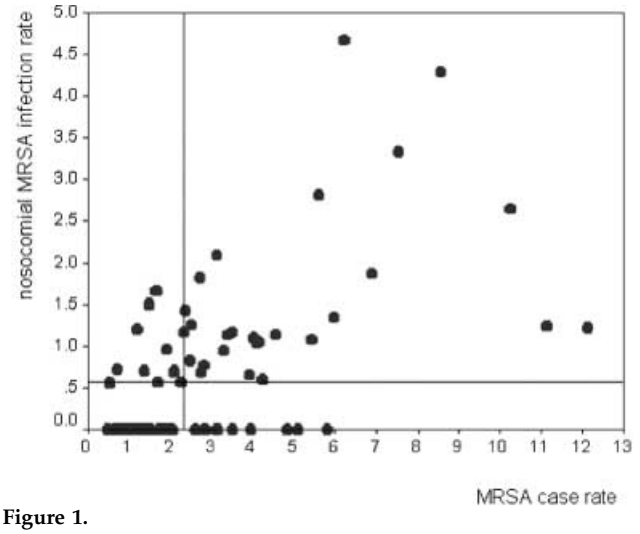

Figure 1.

Conclusions: The distribution of ICUs in Figure 1 allows one to distinguish ICUs with a low nosocomial MRSA infection rate despite having a high MRSA case rate (upper left quadrant) from those with a low nosocomial MRSA infection rate in spite of a high MRSA case rate (lower right quadrant). Comparison of the infection control measures within these two types of ICUs may contribute to stopping the spread of MRSA inside ICUs.

O192 Pseudo-outbreak of *Stenotrophomonas maltophilia* bacteraemia related to non-sterile blood collection vials

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Objective: Disclose the source of a pseudo-outbreak of *Stenotrophomonas maltophilia* bacteraemia.
Methods: From September 2001 to June 2003, 24 blood cultures collected from patients at a Brazilian pediatric emergency unit (PEU) were positive for *S. maltophilia*. There was not a clinical-laboratory correlation in any cases and the occurrences were restricted to the specific hospital unit. Patient ages varied from 1 to 10 years. Epidemiological investigation included: culture of items used at PEU, extensive data and procedure review, and clonality evaluation of isolates. Cultures of pediatric blood collection vials and various environmental samples were done on sheep blood agar and tryptic soya broth. Clonality was evaluated through enterobacterial repetitive intergenic consensus sequences polymorphism (ERIC-PCR) method and with pulsed-field gel electrophoresis (PFGE) of genomic DNA restriction fragments obtained with SpeI.
Results: Genomic DNA restriction patterns of the first 13 isolates were indistinguishable, which led to an intensive surveillance with no source disclosure at first. As part of data and procedure review, it was detected that blood samples for erythrocyte sedimentation rate (ESR) and for blood cultures were collected during the same venous punction in all events. Cultures of the sodium citrate solution from specific batches of ESR collecting vials were positive for S.maltophilia. Analysis of ERIC-PCR and PFGE genomic DNA restriction patterns from clinical isolates and ESR vials showed that they belonged to the same clone.
Conclusion: Utilization of a non-sterile ESR collecting vial associated with an order inversion of vials during sampling caused the pseudo-outbreak. Traditional epidemiological methods associated with molecular epidemiology techniques were fundamental for establishing the precise cause of the pseudo-outbreak.

O193 Compliance with hand disinfection in intensive care units (ICU): the Hawthorne effect

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Objectives: Hand disinfection (HD) is believed to be the most important means of preventing nosocomial infections. The Hawthorne Effect refers to the tendency of people who are being observed in a research contest to behave differently from the way they otherwise would. When collecting data, on compliance with hand HD the situation ought to be avoided where the personnel observed behave differently from when not observed. An investigation was carried out into what influence the Hawthorne Effect has on HD compliance in health care personnel.
Methods: Medical personnel in five ICUs were monitored in two periods regarding compliance with HD where there were potential opportunities for HD. In the first period the personnel had no knowledge of being observed, as the person monitoring them was present in the ICUs anyway because of other duties. The second observation period was announced to the ICUs in advance with detailed information about what the observer would monitor. Observations were done for 2 h during the morning shifts over 10 days in both periods in all the ICUs. Potential confounders of HD compliance included: occupational groups (nurses, doctors, other health care workers), ICUs, nurse-to-patient ratio at the times of observation. Differences in proportions were compared by chi-square tests.
Results: Data were collected from 2808 situational opportunities for HD, of which 937 were in period one and 1871 in period two. Of the staff categories nurses had contributed 57% of all the opportunities, doctors 18% and other health care workers 25%. Altogether in the first period the compliance was 29% and in the second period 45%. Nurses had a compliance of 30 and 58% and doctors a compliance of 25 and 47%. The differences between first and second periods were significant $P < 0.001$. Other health care workers had a compliance of 29 and 21%. This difference was not significant.
Conclusion: In the first period substantially fewer HD procedures were observed with the observer working covertly. Nurses and doctors had a significantly higher compliance in the second period with the observer working overtly. The less variable compliance in the group of other health care workers is explicable as they are not regular workers at the ICU and were not informed about the observation. The Hawthorne Effect has a marked influence on the HD compliance. HD compliance may be lower as we thought because of results from studies which did not regard the effect.

Communicable infections: surveillance systems and public health issues

O194 Assessing cases for SARS in the Netherlands

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Objectives: To evaluate the assessment of cases presented under suspicion of SARS and to estimate the impact of various case definitions.
Methods: Descriptive study of the cases submitted between 17 March and 7 July. A two sources capture-recapture method was used. The first source consisted of cases reported by clinicians and general practitioners to community health services. Public health physicians submitted the cases for further assessment to the National Co-ordination Centre for Communicable Diseases (LCI). The other source consisted of the data base from the Reference Laboratory for SARS (Virology Dept, Erasmus MC) where clinical specimens were received. The first assessment by clinician/public health physician was compared with the assessment on the basis of the ruling case definition at that moment and with a retrospective reassessment using the latest Dutch case definition (i.e. WHO-case definition, 1 May 2003).
Results: In the Netherlands five case-definitions were issued, reflecting the dilemmas with respect to specificity versus sensitivity within risk assessment. Seventy-two patients were submitted for SARS assessment of which 51 to the LCI, 37 to the reference laboratory; 16 patients were identified in both data bases. The three major criteria for SARS (respiratory disease, fever > 38°C and travel related risk) were met in 25 cases (34%). Using the most sensitive case definition (i.e. WHO; revised 1 May 2003) 21 cases would have required reporting for SARS-assessment as suspect cases and two patients as probable cases. Using the more specific case definitions, only nine cases would have met the criteria for reporting and SARS assessment, whereas, despite the case definitions, 52 cases were presented as serious suspect by clinician or public health physician. Risk ratios for being a suspect case were calculated comparing the presentation by the physician versus the first assessment and the reassessment. The reference laboratory performed serological testing for antibodies against SARS-CoV, as well as virus culture and PCR. None of the patients tested positive for the SARS-CoV.
Conclusions: Even when using case definitions with a high specificity over-reporting of cases takes place. Over-reporting of cases should be taken into account when estimating and planning epidemic control resources. The non-cases would require a thorough assessment and case management as well. A centralised assessment centre is recommended.

O195 The current health and economic burden of varicella in Germany

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Objectives: In the absence of any vaccination strategy, nearly every person will suffer from varicella resulting in an estimated 739 000 cases each year in Germany. Varicella can lead to severe complications and even death. Up to now the German vaccination strategy has targeted risk groups only. The aim of this study is to estimate the health and economic burden of varicella under this vaccination strategy.
Methods: The validated simulation model Economic Varicella Vaccination Tool for Analysis (EVITA) (Banz et al. 2003) in which vaccination strategies can be defined by targeted age-group and coverage rate was used to estimate the burden of varicella under the risk group vaccination strategy over a period of 30 years. Input data for the model came from a representative epidemiological survey of 1334 varicella cases collecting data on the course of disease (complications, hospitalisation) and economic data (Wagenpfeil et al. 2004) and a representative seroprevalence survey analysing sera from 4602 persons (Wutzler et al. 2002). Based on the number of varicella vaccine doses sold in the German market, a coverage rate of 10% in the risk group of adolescents aged 12–15 years has been assumed.
Results: Under the vaccination strategy, 721 400 varicella cases occur on average each year in Germany. Thereof, 38 700 cases result in complications which are distributed among bacterial superinfections (45.1%), pneumonia/bronchitis (18.6%), otitis media (16.1%), acute neurological disorders (2.6%) and others (17.6%). 5500 complications lead to hospitalisations because of bacterial superinfections (6.1%), pneumonia (69.1%), acute neurological disorders (8.0%) and others (16.8%). Overall, 21 deaths are

caused by varicella p.a. Varicella and varicella vaccination leads to overall costs of €166.9 million p.a. to society (78.2% work loss costs, 20.5% treatment costs, 1.3% vaccination costs). Thereof, €72.7 million are paid by sickness funds (50.4% work loss costs, 46.5% treatment costs, 3.1% vaccination costs).
Conclusions: Varicella causes a high medical burden of disease which cannot be influenced significantly by the risk group vaccination strategy. Varicella causes also a considerable economic burden to society and health care payers while vaccination costs are very low. The high disease burden speaks for the introduction of more effective universal vaccination strategies, e.g. targeting at young children.

O196 StAKoB – The German working group of centres for the management of highly contagious diseases as part of a preparedness network responding to biological hazards

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Objective: Bioterrorism (BT) attacks and biological warfare (BW) are nowadays recognised as potential threats, which may encounter any country of the world. Pathogens implicated in such events are similar to those, which are eventually imported by single persons. To increase readiness for the treatment of highly contagious diseases Germany has implemented eight centres of competence throughout the country. In 2003 these centres launched the German working group of centers for the management of highly contagious diseases (StAKoB).
Methods: The usefulness of StAKoB and its centres for the preparedness in the case of BT attacks and fitting into existing scenario planning is evaluated.
Results: Recommendations for the management of single or a few cases suffering from highly contagious disease are available in many countries as so in Germany. Statements regarding mass treatment by providing adequate care for a large number of victims in classified containments apart from influenza pandemic plans and smallpox vaccination programmes are usually not given. StAKoB group members have developed recommendations for the post-exposure prophylaxis, mass treatment and containment of considerable quantities of patients. Additionally, training courses for non-medical and medical first responders were installed as well as arrangements for mass quarantine. Harmonizing individual center approaches resulting in generally accepted procedures facilitated the use of personal protection materials as well as the exchange of personnel from each centre.
Conclusions: StAKoB activities and its members are adding favourably to federal preparedness plans.

O197 Evidence of shiga-like toxin (stx1, stx2), intimin (eaeA), and haemolysin (hlyA) positive *E. coli* in private and public drinking water supplies in Upper Austria – an emerging issue of public health?

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Objective: To evaluate the presence of shiga-like toxin (stx1 and stx2), intimin (eaeA), and haemolysin (hlyA) genes in *E. coli* isolates from private and public drinking water supplies in Upper Austria and to assess a possible public health risk. It is well known that consumption of food or potable water containing shiga-like toxin producing *E. coli* isolates can lead to haemorrhagic diarrhoea and even to haemolytic uremic syndrome. Outbreaks associated with public water supplies are well documented (Olsen et al. 2002; Bopp et al. 2003).
Methods: Samples were taken from 255 private and public drinking water supplies in the western part of Upper Austria. This region is mainly supplied by wellwater and springwater. The samples were part of the routine surveillance programme according to the regulation concerning water intended for human use of the Austrian Health Authorities based upon the guideline 98/93 of the European Union. Of the 255 samples 102 were drawn from drilling wells, 86 from dug wells, 54 from springs and 13 from water supplies not otherwise specified. The samples were taken according to standardised procedures. Hundred millilitres of each water sample was processed by membrane filtration. Endo-Agar was used to screen and isolation was performed on Chromogenic-Agar to yield colonies. Presence of *E. coli* genes was assessed by PCR according to published methods.
Results: 11 EaeA containing samples were found in all sorts of water supplies; the 3 HlyA positive *E. coli* were from one drilling well and two springs. Most importantly three shigatoxin 2-containing samples (one of which with additional stx1 gene) were discovered in one drilling well, one dug well and one spring.
Conclusion: The evidence of shiga-like toxin (stx1 and stx2), intimin (eaeA), and hemolysin (hlyA) genes in *E. coli* isolates of 17 of 255 public and private water supplies in Upper Austria is not unexpected and underlines the importance of regular assessments. True EHEC strains, carrying both shigatoxin (stx1 or stx 2) and virulence genes (eaeA or hlyA), however, were not found. Efforts should be continued to guarantee the supply of the public and the private with potable water according to the guideline 98/93 of the European Union.
Literature:
Bopp *et al. J Clin Microbiol* 2003;41:174–180.
Olsen *et al. Emerg Inf Dis* 2002;8:370–375.

O198 Bacterial meningitis in Denmark. Discrepancy between laboratory registered and notified cases

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Objectives: Treatment recommendations for bacterial meningitis (BM) are to a large extent based on notified cases, but notification rates have been reported to be incomplete. We wished to determine the variation in notification rates among cases of verified BM with positive microbiology.
Methods: Cases of BM was identified in the laboratory information systems of 12 of 15 existing Danish departments of clinical microbiology (dpt.cm) in the year 2002 (population served 4 520 000). Cases were included if positive cultures of cerebrospinal fluid were accompanied by clinical and biochemical findings compatible with BM and contamination was effectively ruled out. Patients with positive blood cultures and a diagnosis of BM resting on clinical and biochemical findings were also included. Four dpt.cm supplied data on CSF culture-negative and blood-culture positive cases. Neuro-surgical cases and mycobacterioses were excluded. The National Notification System for Infectious Diseases supplied relevant data. Capture–recapture methods were not performed because of population differences.
Results: A total of 196 culture positive cases of BM were identified in a population of 4 520 000. *S. pneumoniae* constituted 88 cases (45%), *N. meningitidis* 35 cases (18%), *S. aureus* 14 cases (7%), *E. coli* eight cases (4%), *L. monocytogenes* six cases (3%), *E. faecalis* four cases (2%), streptococci 23 cases (12%), *H. influenzae* two cases (1%), miscellaneous bacteria 16 cases (8%). Of these 196 cases, 78 cases (40%) were not notified. The notification rate for *S. pneumoniae* was 88% (77 of 88), for *N. meningitidis* was 97% (34 of 35), for *S. aureus* 7% (one of 14), for *E. coli* 0% (0 of eight), for Listeria 83% (five of six), for *E. faecalis* 0% (0 of four), for streptococci 13% (three of 23), for *H. influenzae* 50% (one of two), and for misc. bacteria was 25% (four of 16).
Conclusion: Notification rates among cases of BM varies according to bacterial aetiology. If recommendations for empirical treatment is based on notification data only, there is a risk of insufficient initial antibiotic treatment for underreported bacterial agents.

O199 Results from the first 6 months of enhanced surveillance of severe *Streptococcus pyogenes* disease in England and Wales

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Objectives: Analyses from the first 6 months of enhanced surveillance of severe *S. pyogenes* infections in England and Wales, part of the EU FP-5 funded 'strep-EURO' programme, were undertaken to determine the burden, clinical presentation and risk factors for disease, and microbiological characteristics of strains.
Methods: Two sources were used to identify cases: routine surveillance of microbiologically-confirmed *S. pyogenes* infection reported to the Communicable Disease Surveillance Centre; isolate referral to the national *Streptococcus* & Diphtheria Reference Unit. Reconciled data from both systems were analysed and described.
Results: A total of 1055 confirmed cases of severe *S. pyogenes* disease were received between 1 January and 30 June 2003, a 6-monthly incidence of 2.01/100 000 population. Of these, 51% were in men, 10% in children (<15 years) and 31% in young adults (15–44 years). Eighty-nine per cent (942) had positive blood cultures, with 10% (105) diagnosed from other sterile sites. In eight cases, sterile site isolates were not reported, but because of their severe and characteristic disease presentation met the case definition. Serotyping results indicated M3 to be the most common type. Analysis of survey questionnaires entered to date indicated that 93% (433 of 455) of cases presented with bacteraemia, 8% (35) with streptococcal toxic shock syndrome, 9% (39) with septic arthritis, 6% (27) with pneumonia, 5% (21) with necrotising fasciitis, 3% (12) with puerperal sepsis and 1% (6) with meningitis. Twenty-one per cent of cases were admitted to intensive care units. Of cases whose outcome was reported (86%), over one-fifth (22%; 88 of 393) had died within 7 days of the microbiological diagnosis. Only in 2% of these was the infection not thought to have contributed to the patient's death, the majority (80%) being directly attributed to the *S. pyogenes* infection. In 22% (97 of 436) of cases, no predisposing risk factors were identified. Of the remainder, injecting drug use was the most common (21%; 91 of 436).
Conclusion: Preliminary results from enhanced surveillance suggest the incidence of severe *S. pyogenes* disease in England and Wales to be considerably higher than previously estimated. Injecting drug use appears to have become a major risk factor for severe *S. pyogenes* disease. Early results affirm the high and rapid mortality associated with these diseases, one-fifth of cases having died within 7 days of initial diagnosis.

O200 *Streptococcus pyogenes* isolates resistant to ciprofloxacin in Spain: clonal diversity and appearance of ciprofloxacin-resistant epidemic clones

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Objectives: The increasing use of fluoroquinolones for the treatment of bacterial respiratory infections has led to the emergence of *S. pyogenes* isolates with resistance to this class of antibiotics. In this study, we determined the mechanisms of ciprofloxacin resistance and the emm-types associated to ciprofloxacin resistance in Spain.
Methods: *S. pyogenes* pharyngeal isolates belonged to the SAUCE (standing for Susceptibility to the Antimicrobials Used Commonly in 'España' and is the Spanish word for the willow tree) national surveillance collection (1998–99). Sequence analysis of specific PCR products was used to deduce emm-types, and to detect mutational alterations in the QRDRs of gyrA and parC. Sequencing was done with the Big Dye terminator mix and autosequenator (Applied Biosystems). DNA sequences were subjected to homology searches against the bacterial DNA database. MICs to ciprofloxacin were determined with and without reserpine following standard procedures. Genetic diversity of the *S. pyogenes* ciprofloxacin resistant isolates was studied by pulse field gel electrophoresis of genomic DNA restricted with SfiI.
Results: All the ciprofloxacin resistant *S. pyogenes* belonging to the SAUCE collection were analysed ($n = 27$). We found point mutations at Ser79 and/or at Asp91 of parC that were replaced in all the strains by Ala and Asn, respectively. By contrast, we did not identify mutations in the gyrA gene in any isolate. In addition, MIC of ciprofloxacin for these isolates (4–8 μg/mL) was not influenced by the presence of reserpine. 66.6 % of the resistant isolates were M type 6 and the rest expressed different M types. PFGE analysis revealed that 50% of the M type 6 had an identical restriction pattern. By contrast we identified up to 12 different PFGE patterns among the rest of the ciprofloxacin resistant strains.
Conclusions: Reduced susceptibility of *S. pyogenes* to ciprofloxacin in Spain is essentially caused by point mutations of parC present mainly in a single M type 6 clone.

O201 External quality assesment of antibiotic susceptibility testing by laboratories participating in the European Antimicrobial Resistance Surveillance System in 2003

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Objectives: The goal of this exercise was to assess if laboratories participating to European Antimicrobial Resistance Surveillance System (EARSS) correctly detect resistance mechanisms that are clinically and epidemiologically relevant and to compare susceptibility test results across countries and guidelines.
Methods: A panel selected six strains: (1) *S. aureus* U2A166 susceptible to relevant antibiotics, (2) MRSA U2A1786 heteroresistant to methicillin and known from outbreaks, (3) *S. pneumoniae* U2A961 resistant to erythromycin, (4) *S. pneumoniae* U2A1787 intermediately resistant to penicillin, (5) *E. coli* U2A1789 producing ESBL (SHV5) 6) *E. gallinarum* U2A604 with VanC type resistance. The strains were distributed by UK-NEQAS (United Kingdom National External Quality Assessment Service) to 740 laboratories which were asked to report clinical categories (S, I, R). Results were analysed and considered 'concordant' if the reported categorisation agreed with the designated interpretation of the reference laboratories.
Results: 673 (90%) Laboratories from 26 countries returned reports in time. For the *S. aureus* U2A166 the concordance was >95% for oxacillin, cefoxitin, gentamicin, vancomycin, teicoplanin and penicillinG. For the MRSA the concordance was low for oxacillin (80%) and cefoxitin (76%), whereas concordance was >94% for gentamicin, vancomycin, teicoplanin and penicillin G. Erythromycin resistance and clindamycin susceptibility of *S. pneumoniae* U2A961 was correctly detected by 95% of laboratories. For the *S. pneumoniae* U2A1787 the concordance for penicillinG intermediate resistance was only 76%. For the *E. coli* the concordance was high for detection of ESBL production (94%), and for gentamicin (99%) and ciprofloxacin (99%). The *Enterococcus* was identified as *E. gallinarum* by 50% of laboratories, but more importantly, 90% of laboratories reported the reduced susceptibility to vancomycin and the susceptibility to teicoplanin, which is typical for this type of resistance.
Conclusions: Participating laboratories were capable of detecting most resistance phenotypes. However, 20% of laboratories missed detection of an MRSA that caused epidemics in Europe. This resistance was difficult to detect as the resistance phenotype was heterogeneously expressed. Moreover, 11% of laboratories failed to detect the reduced susceptibility to penicillin in *S. pneumoniae*. The results illustrate that there is room for improvement in European routine susceptibility testing.

O202 Bacteriologic diagnostics and antibiotic management in German intensive care units: data from project SARI (Surveillance of Antibiotic Use and Bacterial Resistance in German Intensive Care Units)

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Objective: To evaluate bacteriologic diagnostics and antibiotic management in intensive care units (ICUs) in Germany.
Methods: A questionnaire was sent to each of the 38 (3/2003) ICUs participating in project SARI, which was initiated in February 2002. Of these ICUs, 29 returned the questionnaire for analysis. The questionnaire contained questions on the following: implementation of guidelines concerning antibiotic treatment in ICUs, diagnostic procedures and empiric choice of antibiotics given for pneumonia, bloodstream infections, surgical site infections and other community acquired and nosocomial infections in ICUs.
Results: 19 ICUs use written guidelines on antibiotic management. Only 14 of 29 ICUs collect quantitative specimens for ventilator associated pneumonia, although recommended by national guidelines. Empiric antibiotic treatment differs considerably in ICUs, even for common infections. 29 ICUs use nine different first line treatment options for late onset ventilator-associated pneumonia. Only six ICUs favour a reasonable combination therapy to target *P. aeruginosa* and *Acinetobacter* sp. as important underlying pathogens. Resistance rates for *P. aeruginosa* (testing in accordance with DIN) are high in SARI-ICUs: 18.3% for ciprofloxacin, 26.1% for imipenem and 24.3% for piperacillin/tazobactam (cumulative data from 2/2000–6/2003). Regarding empiric central line associated blood stream infection, 26 ICUs use a total of 12 first line treatment options. 18 ICUs (69%) use glycopeptides alone or in combination, in spite of the fact that MRSA rates are low (<10%) in their ICU ($n = 5$).
Conclusion: Although national guidelines are available, considerable differences still exist in microbiologic diagnostics and empiric antibiotic treatment in ICUs in Germany. Guidelines for ICUs have proved to be a useful instrument for quality management and for the improvement of empiric antibiotic treatment and microbiologic diagnostics. However, discrepancies exist between the recommendations given by guidelines and actual medical practice in the ICU setting.

O203 Outpatient systemic antibiotic use in 2002 in Europe

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Objectives: ESAC, European Surveillance of Antimicrobial Consumption, granted by DG/SANCO of the European Commission, is an international network of surveillance systems, aiming to collect comparable and reliable data on antibiotic consumption in Europe. Thirty-two countries joined the ESAC project, including all 15 EU countries, 12 of the 13 applicant countries (not Cyprus), two of the three EFTA-EEA countries (not Liechtenstein), Croatia, Russia and Switzerland. Twenty-six of the 32 participating countries were able to deliver ambulatory care data for 2002.
Methods: Outpatient antibiotic use for 2002 was collected, using the ATC/DD method. Data were expressed as DDD per 1000 inhabitants per day (DID). Detailed information on the sources of antibiotic use data can be found at the ESAC website (http://www.ua.ac.be/ESAC).
Results: Antibiotic use varied with a factor of 3.6 between the countries with the highest and lowest consumption (32.2 DID in France and 9.0 DID in the Netherlands). Penicillin (J01C)(PEN) use varied with a factor of 4.7 between France (16.3 DID) and the Netherlands (3.5 DID). We observed that in three countries (Norway, Sweden and Denmark) the narrow spectrum PEN (J01CE) still represented more than 60% of the PEN, whereas in seven countries (Belgium, France, Italy, Latvia, Luxemburg, Portugal and Spain) these drugs represented <2% of the PEN. Cephalosporin (CEP) (J01DA) use varied with a factor of 256.2 between Greece (6.7 DID) and Denmark (0.03 DID). In France and Italy, the high CEP use was because of the markedly high use of third generation CEP, representing about one-third of CEP use in these countries, i.e. of the injectable (ceftriaxone in Italy) and oral (ceftibuten and cefixime in Italy; cefpodoxime and cefixime in France) CEP. Total use of the macrolides (J01FA), lincosamides (J01FF) and streptogramins (J01FG) varied with a factor of 26.9 between Greece (7.8 DID) and Latvia (0.3 DID). Quinolone (J01M) use varied with a factor of 21.2 between Italy (3.76 DID) and Denmark (0.17 DID). Sulphonamides and trimethoprim (J01E) use varied with a factor of 19.5 between Iceland (2.0 DID) and Latvia (0.1 DID).
Conclusion: Diverging patterns in antibiotic consumption were observed which need to be confronted with the nature and intensity of health policy measures.

Emerging infectious diseases

O204 Profile of antibody responses against SARS-coronavirus recombinant proteins and their potential use as diagnostic markers

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Objectives: A new coronavirus, SARS-CoV, has been identified to be the aetiological agent of severe acute respiratory syndrome (SARS). Given the highly contagious and acute nature of the disease, there is an urgent need to develop diagnostic assays that can detect SARS-CoV infection. The aim of this study is to determine which of the viral proteins encoded by the SARS-CoV genome may be exploited as diagnostic antigens for the development of serological assays.
Methods: In this study, several proteins encoded by the SARS-CoV were expressed in mammalian and bacterial cells and tested for their immuno-reactivity with sera from SARS-CoV-infected patients using Western blot analysis. The bacterially expressed proteins were also used to develop a SARS ELISA Test and a 15-min immunochromatographic device, SARS Rapid Test. In addition, an immunofluorescence method ulitizing mammalian cells stably expressing the heavily glycosylated spike (S) protein, is used to test for antibodies against S.
Results: A total of 83 sera, including six from convalescent patients, 63 from probable patients who were discharged from the hospital and seven pairs from two different time-points of infection, were analysed by Western blot analysis. All of them showed immuno-reactivity towards the nucleocapsid (N) protein of SARS-CoV. Sera from some of the patients also showed immuno-reactivity to a protein that is unique to SARS-CoV, U274. The results obtained using the SARS ELISA Test and the SARS Rapid Test were consistent with that obtained using Western blot analysis. In addition, all the convalescent sera showed immuo-reactivity against the spike (S) protein. However, samples from the acute phase (2–9 days after onset of illness) did not react with S, suggesting that antibodies to N may appear earlier than antibodies to S or this could be due to the difference in the sensitivities of the two methods. The immuno-reactivities to these recombinant viral proteins are highly specific as sera from 100 healthy donors did not react with any of these proteins.
Conclusion: The recombinant N and U274 proteins may be used as antigens for the development of serological assays for SARS-CoV. For detecting of anti-S antibodies in patient's serum by

immunoflourescence, the stable cell-line expressing S would be safer to use than SARS-CoV infected Vero cells, which are currently being used in serological assays.

O205 Recombinant N and M protein-based ELISA for detection of antibodies to SARS-Coronavirus

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Objectives: A new Coronavirus (SARS-CoV) is considered the aetiological agent of the Severe Acute Respiratory Syndrome (SARS). Molecular, virologic and serologic tests have been developed for its identification, the latter currently based on purified virus or infected cells. In this study we report the development of an in-house ELISA based on recombinant SARS CoV proteins, the nucleoprotein N and the membrane protein M.
Methods: The coding region of N and M were cloned and expressed in *Escherichia coli*. Moreover the NH2-terminus, central-portion and COOH-terminus of the N protein, and the cytoplasmic tail of the M protein, were also cloned and expressed. The purified proteins were used as coating antigens in an in-house ELISA. Six reference sera from SARS patients with different immunofluorescence and virus-neutralisation titres and 93 sera from healthy or no SARS patients with low tract respiratory infections were tested.
Results: High-titres of IgGs were detected in all sera from SARS patients, whereas no reaction was detected in the 93 control sera. The most discriminative and reproducible ELISA results were obtained with the cytoplasmic tail of the M protein (M2), as antigen. Moreover in this condition the six positive sera examined, showed almost the same values of O.D. at each dilution tested.
Conclusions: While the low number of SARS sera examined would preclude a sound assessment of sensitivity of the ELISA methods we generated, the specificity of the assay appears to be rather high considering the elevated number of negative sera tested. Overall, our results strongly suggest the effectiveness of recombinant antigen-based in house ELISA for serological diagnosis of SARS, in particular when the novel M2 antigen is used.

O206 Plague returns to Algeria after an absence of 50 years

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Background: Throughout the last century, imported cases of plague regularly occurred in Algeria. To date, no natural focus of plague had been described in this country. The last human cases were reported in Oran in 1950. On 23 June 2003 the Algerian authorities reported 10 cases of bubonic plague to the World Health Organization (WHO) and sought technical support to respond to this outbreak.
Methods: A team of five experts from WHO assessed the situation and supported the national authorities in containing the outbreak. Strains were isolated from human specimens and typed by the Institut Pasteur, Paris, France.
Results: On 5 June 2003, a child from a village 25 km south of Oran, died suddenly with a severe infectious syndrome. During the following weeks a total of 11 confirmed and seven suspected cases of plague were reported from the same area. On 20 June, the bacteriological analysis performed at the University Hospital in Oran confirmed the diagnosis of plague. The last case was reported on 17 July. All the cases were bubonic plague, with two cases having evolved into the septicemic form. No human-to-human transmission was observed. Only one death, the first case, occurred. The outbreak was controlled by a variety of means: hospitalization and treatment of patients; chemoprophylaxis of the contact cases; vector control and improvement of general hygiene. Molecular typing of the isolates indicated that they belonged to the most common *Y. pestis* type (biovar Orientalis, ribotype B) and that all five strains were clonal.
Conclusion: The first observations are compatible with the hypothesis of the reactivation of an autochtonous focus in the region of Oran, but in-depth field investigations will be necessary to confirm this hypothesis. The return of plague to Algeria 50 years after its last occurrence, near an international harbour, emphasizes the need to maintain thorough epidemiological surveillance at the international level.

O207 *Corynebacterium ulcerans* diphtheria in France; a dog as the culprit

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A 47-year-old woman with typical laryngeal diphtheria was admitted to Bicêtre hospital (France) for severe dyspnoea. Throat swab grown on Loeffler's medium and blood agar gave of a gram-positive catalase positive-rod identified as *Corynebacterium ulcerans* by positive urease reaction, API Coryne system and sequencing of 16 sRNA gene. Disk diffusion test showed that this isolate was susceptible to most antibiotics including beta-lactams and macrolides. A PCR test was positive for the gene of diphtheria-like toxin that is usually quite invariable in *Corynebacterium diphteriae*. Sequencing identified a gene that encoded a toxin that had twenty-five amino acid substitutions as compared with diphtheria toxin. The patient was treated with amoxicillin-clavulanic acid (before identification of *C. ulcerans*) for 5 days, then penicillin G adapted to renal function for nine days and one dose of diphtheria antitoxin (40 000 U). She recovered without complication. She was immunocompromised (prednisone 6 mg/day, tacrolimus 12 mg/day, and mycophenolate nofetil 1 g/day) as she had underwent kidney graft and a retrospective analysis did not evidence detectable diphtheria antibodies in her serum. Carriers of *C. ulcerans* in the immediate vicinity of the patient (family, patients and health care workers, $n = 88$) using a novel selective medium settled for this purpose [Columbia colistin-nalidixic acid (CNA) agar plates with 5% sheep blood plus fosfomycin 125 mg/L] were not identified. However, the exact same *C. ulcerans* isolate was found from throat, nostrils and labial ulceration of the companion dog of this patient. This dog had chronic cutaneous ulcerations, sneezing and rhinorrhea for years. Failure of an amoxicillin-containing treatment (2 g/day for 15 days) for eliminating the bacteria from the dog lead to his killing. Although the main natural reservoir of *C. ulcerans* is cattle and cattle environment, this is the first report that suggests transmission from a dog to human of a coryneform bacteria as a source of diphtheria. In addition it raises the growing question of pets as a reservoir of zoonotic infection in immunocompromised patients.

O208 Group B *Streptococcus* necrotising fasciitis: an emerging disease?

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Objective: We report our recent observation of five cases of Group B *Streptococcus* (GBS) necrotizing fasciitis in our patients.
Methods: The patients were all female, age ranged from 38 to 66 years. Four patients had poorly controlled diabetes mellitus as the predisposing condition. Four cases involved the lower limbs and one involved the hand. GBS was the only organism isolated in the tissue culture in all cases and in the blood culture in one patient. The GBS isolated had identical antibiogram with all being susceptible to penicillin. One patient fulfilled the definition of streptococcal toxic shock syndrome. Two patients had previous soft tissue infections where GBS were isolated prior to the necrotizing fasciitis episodes. All five patients survived with

appropriate antimicrobial use and early aggressive surgical débridement.
Results: Single-organism necrotizing fasciitis caused by GBS is extremely rare. To date, only over 10 cases were reported in the English literature. Together with the five cases we observed, it is clear that group B *Streptococcus* is capable of causing necrotizing fasciitis. While the virulence factors that enable GBS to cause necrotizing fasciitis have not been established, this report highlights the emergence of this clinical entity.
Conclusion: The medical literature has focused on GBS as a disease of pregnancy and neonatal period. While, the emergence of invasive GBS in adults has recently been high-lighted, awareness should be raised on the spectrum of soft-tissue infections caused by GBS, in particular the danger of progression to necrotizing fasciitis. This emerging phenomenon afflicts particularly diabetics. Awareness of this disease should heighten the index of suspicion when treating susceptible patients with GBS soft-tissue infections. Our small case series add to the limited available literature underscoring the potential for GBS to cause necrotizing fasciitis. Early recognition, aggressive surgical débridement and high dose intravenous penicillin therapy are the cornerstones in the management of this deadly disease.

O209 An emerging infection in Turkey: Crimean Congo haemorrhagic fever

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Objective: To describe the epidemiology and clinical features of an outbreak detected for 2 years in Turkey.
Method: During springs summers 2002–03, clusters of cases with similar symptoms and laboratory findings have been detected in Middle Black Sea and Northern part of Mid Anatolia. The patients, families, and the doctors have been interviewed with a questionnaire. Although, Q fever was considered as the clinical diagnosis in 2002, according to the clinical and epidemiological findings next year, a viral aetiology was suspected. Laboratory tests included Q fever detection by IFA and CCHF investigation by ELISA (IgG/IgM)/ RT-PCR on the S segment of the viral genome and sequence analysis. The presence of CCHF IgM antibodies and/or detection of viral genome by RT-PCR indicated an acute CCHF case.
Results: In 2002, seven of 19 cases (36.8%) were positive for Q fever by IFA. From April to September 2003, a total of 155 serum samples were received from 128 patients from 15 provinces in which 45.3% from the same province. All the specimens for suspected cases tested for Q fever were negative. Acute CCHF infection was recorded in 86 patients (67.2%): 82 cases with presence of CCHF IgM antibodies (in association with positive RT-PCR in 18 cases) and four cases with positive RT-PCR positive. Therefore CCHF IgM and IgG antibodies were reported in 49 of 116 patients (42.2%). Retrospective analysis on specimens from the year 2002 indicated that 17 of 26 (65.4%) patients were CCHF cases. Frequent clinical symptoms in confirmed cases included were fever 86.4% (57 of 66) arthralgia/ myalgia 77.5% (31 of 40), nausea 77.3% (51 of 66) and vomiting 68.2% (45 of 66). Haemorrhagic features were unfrequently recorded: petechial rash 22.7% (15 of 66), epistaxis 22.7% (15 of 66), intestinal bleeding 9.1% (six of 66), haemoptysis 4.9% (two of 41). Thrombocytopenia 96.8% (61 of 63), leukopenia 90.6% (58 of 64), elevated AST 98.2% (56 of 57) and ALT 94.6% (53 of 56) levels were the main laboratory findings. Fatality rate was 8.1%. Among risk factors, tick contacts have been detected in 72.5% (29 of 40) of the cases. Intra-familial cluster of cases or nosocomial infections were not reported.
Conclusion: This is the first confirmation of the presence of CCHF virus in Eastern Turkey since 1980 when CCHF serological evidence was reported. The numerous infections in human in 2003 suggest to consider CCHF as a main public health concern in direct relationships with tick contact.

O210 Changing microbiological profile of infective endocarditis in Greece during the last 17 years

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Objectives: To evaluate epidemiological trends on microbiology of infective endocarditis (IE) in Greece during the last 17 years.
Methods: Isolates from blood cultures of IE and their susceptibility patterns were constantly recorded in all patients with native valve endocarditis (NVE) and prosthetic valve endocarditis (PVE) via a data base electronic registry form activated in 15 General hospitals of Athens.
Results: 150 NVE and 40 PVE were recorded since 2001 by the Hellenic endocarditis study group. *Streptococcus* spp predominated in NVE (36%) and methicillin resistant Staphylococci in PVE (37.5%). Compared with epidemiological data of 134 IE registered between 1986 and 1995 mainly in our Department, Streptococci still predominates NVE but the incidence of enterococcal endocarditis raised from 5 to 8% in the 80s and 90s to 18% in 2001–03. Staphyloccocal NVE declined by 10% between 80s–90s and 2001–03. Blood culture negative PVE increased to 20%. The incidence of Gram-negative, fungal, *Brucella* and ricketsial endocarditis has not substantially changed.
Conclusions: Microbiological patttern of IE has changed within 17 years. Enterococci in NVE and methicillin resistant Staphylococci in PVE have emerged. Knowledge of current microbiological trends is essential especially for the empirical treatment of culture negative IE. Further investigation in order to establish correlations between the observed microbiological data and other epidemiological factors is required.

O211 New acute tick-borne rickettsiosis caused by *Rickettsia heilongjiangensis* in the Russian Far East

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Objectives: Russian Far East is a geographical, economical and political unit within the Russian Federation. Since 1932 the cause of acute tick-borne spotted fever here is thought to be *Rickettsia sibirica* and its antigen has been used for serological studies in clinical laboratories, although seasonal morbidity, age of affected personals and seropositivity rate (with *R. sibirica* as antigen) differ significantly from correspondent data from other regions where multiple strains of *R. sibirica* have been isolated. The aim of this study was to identify the cause of an acute febrile tick-transmitted disease in the Russian Far East by molecular and serological methods and to describe the clinical picture in these cases.
Methods: We have studied DNA extracted from blood and skin biopsies of 65 acutely ill tick-bitten patients in the year 2002. Nested PCR for gltA gene with modified primers was used to amplify full-length gene. Both upstream and downstream to the tandemly repeated region portions of ompA gene and full ompB have been amplified in the samples positive for gltA. All amplicons has been sequenced. Patients' sera were tested with different rickettsial antigens by IFA.
Results: Amplified and sequenced four portions of three rickettsial genes from the samples of 13 patients showed that these genes belong to *Rickettsia heilongjiangensis* recently isolated from *Dermacentor sylvarum* ticks in the nearby to the Russian Far East region of China. This rickettsia belonging to subgroup of *Rickettsia japonica* was previously suggested to be pathogenic for humans on the base of serological findings. We tested 11 patients' sera with different rickettsial antigens, and confirmed the increasing of IgG and IgM titres to spotted fever group rickettsiae, including *R. heilongjiangensis*. Clinical and epidemiological data on these patients shows that this disease is close to other tick-borne rickettsioses.
Conclusion: Amplification of three different rickettsial genes and serological data suggest *R. hilongjiangensis* as a cause of acute tick-borne rickettsiosis in the Russian Far East. Previously this disease,

probably, has been misinterpreted as Siberian tick typhus. Molecular biology techniques allowed identification of the aetiology of this acute tick-borne disease.

O212 *Laribacter hongkongensis* in fish is associated with gastroenteritis and traveller's diarrhoea

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Objectives: After its discovery in Hong Kong in 2001, *Laribacter hongkongensis* was recovered from six patients with gastroenteritis. Three patients were from Hong Kong, and three from Switzerland. However, the association of *L. hongkongensis* with gastroenteritis is still unproven and the source of *L. hongkongensis* is unknown. We conducted a territory-wide, multi-centred, prospective study to evaluate the association of *L. hongkongensis* with community-acquired gastroenteritis and investigate the epidemiology, clinical features, outcome, and risk factors of patients that were culture positive for *L. hongkongensis*. Targeted food surveillance was performed to identify the potential reservoir of *L. hongkongensis*.
Methods: Faecal samples from patients with community-acquired gastroenteritis and controls were cultured for *L. hongkongensis*. A case–control study and targeted food surveillance were performed to identify the potential source of *L. hongkongensis*. All *L. hongkongensis* isolates were characterised by pulsed-field gel electrophoresis (PFGE) and ribotyping.
Results: During a 4-month period, *L. hongkongensis* was recovered from 17 of 3788 patients with community-acquired gastroenteritis, but none of 1894 controls ($P < 0.005$). Patients that had community-acquired gastroenteritis and were culture positive for *L. hongkongensis* were associated with recent histories of travel (59% vs. 6% in controls, $P < 0.001$), fish consumption (94% vs. 56% in controls, $P < 0.01$), and minced freshwater fish meat consumption (29% vs. 3% in controls, $P < 0.05$). Twenty-seven additional *L. hongkongensis* isolates were recovered from intestinal samples in 25% of freshwater fish (29% of mud carp, 59% of grass carp, 53% of bighead carp and 6% of large-mouth bass) and 15% of minced freshwater fish meat from retail markets in Hong Kong. *L. hongkongensis* of the same PFGE pattern and ribotype was recovered from a patient and minced freshwater fish meat from the retail market where he had recently purchased minced freshwater fish meat for cooking. This particular combination of PFGE pattern and ribotype was not seen in any other isolates.
Conclusion: *L. hongkongensis* is associated with community-acquired gastroenteritis and traveller's diarrhoea. Freshwater fish is the source of *L. hongkongensis*.

O213 Diarrhoea in Danish children under 5 years of age: a case–control study

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Objectives: To clarify the most important aetiologies of infectious diarrhoea in Danish children under 5 years of age.
Methods: Stools from 424 cases with diarrhoea from all over Denmark and 870 healthy age-matched controls were examined for parasites, *Salmonella, Campylobacter, Yersinia, Shigella* and *Vibrio* spp. using standard methods. Stools were also examined for verocytotoxin-producing *E. coli* (VTEC), attaching and effacing *E. coli* (A/EEC) including enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), and enteroaggregative *E. coli* (EAggEC) by colony hybridisation of virulence genes and serotyping. Rota-, adeno- and astroviruses were detected by ELISA and noroviruses by PCR.
Results: Overall, a potential pathogen was found in 57% of cases. The following pathogens were associated with disease with figures given for patients and controls, respectively. Rotavirus: 13% vs. <1%; *Salmonella*: 5% vs. <1%; noroviruses: 5% vs. 2%, adenovirus: 4% vs. <1%; *Campylobacter coli/jejuni*: 3% vs. <1%; EPEC 3% vs. <1%; VTEC 3% vs. <1%; *Yersinia*: 2% vs. <1% and *Cryptosporidium:* 2% vs. 0%. A/EEC (eae positive non-classical EPEC serotypes) were found with equal frequencies in patients (11%) and controls (12%). No *Shigella, Vibrio* or ETEC were isolated.
Conclusions: Rotavirus was the single most common pathogen isolated. *Salmonella* was the most common bacterial pathogen detected in diarrhoea both acquired in Denmark and abroad. VTEC was an important cause (3%) of diarrhoea. A/EEC were equally common in patients and controls, in contrast to EPEC, which was associated with diarrhoea.

Macrolides and inflammation modulation in respiratory tract infections (Symposium arranged by Pliva)

S226 Immunomodulatory effects of macrolides

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Macrolides are widely used as antibacterial drugs. In addition to their antibiotic properties there is a number of reports describing other potentially useful effects. Anti-inflammatory properties can partially be linked to the unique cellular pharmacokinetics of macrolides, particularly to their extraordinary uptake by granulocytes and monocytes. 14- and 15-membered macrolide effects will be discussed at three different levels: *in vitro* experiments, animal models, and finally clinical studies. Considerable evidence, mainly from *in vitro* studies suggests that leucocytes and neutrophils in particular, are important targets for modulatory effects of macrolides on host defence responses. Macrolide antibiotics in vitro regulate different cell functions, e.g., degranulation, oxidative burst, cytokine/chemokine synthesis/secretion, adhesion, chemotaxis, apoptosis, and NO production. Molecular mechanisms of anti-inflammatory action seems to be based on inhibition of transcription factors activity (nuclear factor kappa B and AP-1). Nevertheless, recent microarray-based data on the influence of erythromycin and clarithromycin on epithelial cells are not supportive of this hypothesis. Macrolide antibiotics have also been proven to be effective in animal models of inflammation, e.g. carrageenin pleurisy, zymosan peritonitis, LPS-induced inflammation and immune complex lung inflammation. The most valuable lesson about macrolide potential for treatment of inflammatory diseases has been obtained from the long history of successful therapeutic use of erythromycin and azithromycin in diffuse panbronchiolitis. Some recent trials are indicative of possibility of their long-term use in cystic fibrosis and some other (pulmonary) diseases. Low toxicity and good safety profile of macrolides additionally favour their long-term use. The consequences of anti-inflammatory effects of macrolides in infection, as compared with (chronic) inflammation that is not caused by bacteria are probably different which is something that needs to be considered when developing either new macrolide antibiotics or macrolide anti-inflammatory drugs.

S227 Biphasic modulation by azithromycin of human neutrophil function: implications for therapy

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Modulatory effects of macrolide antibiotics on leucocyte function have been observed by a number of investigators. Relatively few studies have been performed, however, with azithromycin and its effects on leucocytes *in vitro* have been confusing. As the ability of azithromycin to accumulate in leucocytes is much greater than that of other macrolides, a study was performed to investigate the effects of a standard 3-day treatment with azithromycin (500 mg/day, p.o.) on a variety of leucocyte functions and circulating mediators in 12 healthy volunteers. Blood was taken 1 h before treatment, 2.5 h, 24 h and 28 days after the last dose. An initial neutrophil degranulating effect of azithromycin was reflected in rapid decreases in azurophilic granule enzyme activities in cells and corresponding increases in serum. The oxidative response to a particulate stimulus was also acutely enhanced. These actions were associated with high plasma and neutrophil drug concentrations. It seems likely that this acute stimulation of leukocyte function, also observed in some *in vitro* studies, may facilitate anti-bacterial effects of azithromycin. A continuous fall, in response to azithromycin treatment, in chemokine and interleukin-6 serum concentrations, within the non-pathological range, was accompanied by a delayed down-regulation of the oxidative burst and an increase in apoptosis of neutrophils up to 28 days after the last azithromycin dose. Neutrophils isolated from blood at this time point still contained detectable drug concentrations. This delayed, potentially anti-inflammatory activity may curtail deleterious inflammation during recovery from an infection. In a more recent study, using the same azithromycin treatment schedule in patients with chronic obstructive pulmonary disease, reductions in circulating leucocytes and in acute phase proteins were found that would appear to support the potential for azithromycin to exert anti-inflammatory effects.

S228 Macrolides and inflammation modulation in the treatment of respiratory tract infections

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The use of macrolides either as monotherapy or in combination with other agents for the treatment of hospitalised patients with community-acquired pneumonia has consistently been associated with decreased mortality, decreased lengths of hospital stay and decreased overall costs. As these benefits occur even when macrolides are co-administered with other agents with atypical coverage, such as fluoroquinolones, it is likely that these benefits are secondary to a mechanism that is separate from their antibacterial properties. As they have been demonstrated to have beneficial immunomodulatory effects in non-infectious chronic inflammatory airway syndromes when dosed chronically, it is possible they may produce similar benefits when dosed for short periods of time for respiratory tract infections as well. In fact *ex vivo* neutrophil research of healthy volunteers administered a standard 3-day course of oral azithromycin have demonstrated a biphasic modulatory effect of azithromycin on inflammation. First azithromycin causes an enhancement of neutrophil degranulation and oxygen burst in response to particulate matter thereby enhancing the endogenous host defense mechanisms. Once bacteria are no longer present, azithromycin induces a curtailment of IL-8 production and stimulates neutrophil apoptosis so that the body can clear these apoptotic neutrophils without spilling their pro-inflammatory substances thereby suppressing any perpetuation of inflammation and minimizing any secondary short- or long-term morbidity. Additional *in vitro* and *ex vivo* data with other macrolides further support these findings as well as the benefits that they also have to offer. Although additional animal and human studies are needed to validate these findings of the short-term biphasic anti-inflammatory benefits of the macrolides, these initial data provide strong support for a non-antibacterial explanation behind the mortality and length of stay benefits demonstrated with macrolide containing regimens for community-acquired lower respiratory tract infections, including pneumonia.

S229 Clinical use of macrolides in the treatment of RTIs: focus on azithromycin

T. File
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Because the macrolide class of antimicrobials is active against key pathogens associated with community respiratory tract infections (RTIs), agents from this class are frequently included in recommendations for treatment of these common infections: including community-acquired pneumonia (CAP), sinusitis, and acute exacerbation of chronic bronchitis. As they are active against the 'atypical' pathogens, they are particularly useful to treat CAP-either as monotherapy for mild infections or as part of combination therapy with a beta-lactam for serious infections. Whereas earlier macrolides have little activity against *H. influenzae*, clarithromycin and azithromycin are more active, with azithromycin being most active. A prolonged T1/2 with convenient once-daily dosing, high intra-tissue concentrations, and better tolerance (less adverse effects) are significant advantages of azithromycin compared with earlier macrolides. Despite reports of increasing resistance of *S. pneumoniae* to the macrolides *in vitro*, the number of published clinical failures due to macrolide resistance appears to be relatively few given the several millions of prescriptions written yearly. Possible explanations include the increased accumulation of the newer macrolides, such as azithromycin, into tissue fluids and macrophages. In addition, the immunomodulating properties of the macrolides offer beneficial effects concerning the host response during RTIs. Numerous randomized clinical trials as well as observational studies have documented the efficacy and safety of azithromycin in the treatment of RTIs. These studies will be reviewed. The positioning of the macrolides in various guidelines for the management of RTIs differ in part related to the variance in local epidemiology and the clinical relevance of resistance. Presently, azithromycin is prominently recommended in North American guidelines for many RTIs.

Toxoplasmosis in the immunocompromised host (Symposium arranged with ESGT)

S232 Toxoplasmosis in the immunocompromised host: epidemiology and diagnosis

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Toxoplasmosis remains, in the immunocompromised host, a life-threatening and quite frequent disease. To study this opportunistic parasitic disease, two groups of immunocompromised hosts should be defined: AIDS patients with 'classical' cerebral toxoplasmosis, and patients with disseminated toxoplasmosis (AIDS or haematological transplant patients). The epidemiology of cerebral toxoplasmic reactivations in AIDS patients has been largely modified by the use of HAART, while its diagnosis still relies mainly on clinical feature, imaging and evolution under treatment. In non-complicated cases, the use of biological tests (serology) is

useful only for the determination of the risk of reactivation, and the search for the parasite in CSF is not of great help for the physician. The epidemiology of disseminated toxoplasmosis (different from clearly defined cerebral toxoplasmosis) is not clearly known. In fact, data are mainly reported from small series or case report whatever the kind of immunosuppression; AIDS or transplant patients. The diagnosis of disseminated toxoplasmosis (reactivation in the transplant recipient, or transmission through the graft) is often difficult due to the fact that the clinical signs are not specific. Thus, in this case, biological methods are of paramount importance to make the diagnosis. *T. gondii* can be detected in all sorts of biological fluids and biopsies, and the clinical value of such result must be discussed. Several biological techniques can be used to make such a diagnosis. If serological test are not always useful, the diagnosis relies on the direct detection of the parasite. This can be done using *in vivo* and *in vitro* cultures (mouse inoculation and cell cultures) and detection of parasitic DNA par PCR. This technique is, to date, the most accurate to affirm the presence of *T. gondii* in blood, CSF, biopsies. However, the facts that no standardised PCR kits for toxoplasmosis are available, and that the pathophysiology is, to date, not completely understood, should lead to careful interpretation of the biological results.

S233 Toxoplasmosis in the immunocompromised patient: pathogenesis and treatment

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Objectives: *Toxoplasma gondii* is an intracellular protozoan parasite causing clinical and latent infection in humans. The incidence varies in different geographical locations depending on cultural habits and climate. Humans are infected orally by oocysts excreted from the host, the cat, contaminating the surroundings or by the ingestion of cysts found in undercooked meat from animals. In immunocompetent individuals, *T. gondii* causes an asymptomatic infection or fever and lymphadenopathy. In immunocompromised patients, it may cause life-threatening infection. Toxoplasmosis after bone marrow transplantation (BMT) is a rare but serious and life-threatening condition. It is usually caused by a reactivation of latent infection but can probably also be transmitted by marrow or blood products. As the incidence is low a centre will not be able to have enough experience to produce updated guidelines on diagnostics or treatment but there is a need for networks.
Methods: The ESCMID Study group on Toxoplasmosis (www.Escmid.org) and its aims is described. A review of the latest reports is described together with a detailed description of a fatal case.
Results: The incidence of toxoplasmois in BMT is estimated to be between 0.3 and 5%. The most frequently involved organs are the central nervous system, lungs and the heart. The infection is disseminated or isolated organs as the brain or the lungs are affected. Postmortem diagnosis is reported to 53% and mortality attributed to the infection was 66%.
Conclusions: Toxoplasma infection represents a rare but often fatal complication in BMT recipients. It contributes however to post-transplant morbidity and mortality in highly endemic areas. A reduction in incidence and an improvement in outcome is accomplished by an increased knowledge which can only be gathered in a network. We need better knowledge of the clincial signs and symptoms as well as of treatment and prophylaxis. Also, improved diagnostics by molecular techniques and quality control of the assays are essential.

European recommendations for antibiotic resistance surveillance (Joint symposium arranged with ESGARS)

S237 Recommendations for data analysis and presentation

G. Cornaglia, W. Hryniewicz, V. Jarlier, G. Kahlmeter, H. Mittermayer, L. Stratchounski, F. Baquero and ESGARS members

The problem of surveillance of bacterial resistance to antimicrobials in Europe has been extensively debated in many documents issued by National Committees and often assuming the value of National Guidelines. However, a comprehensive document addressing the whole matter of antimicrobial resistance surveillance from a European perspective, as well as reviewing its present status and drafting future perspectives, is still lacking, thus ESGARS has committed itself to producing a wide-acceptance document through a consensus process involving all members of the Study Group. This document focuses on the detection of bacterial resistance and its reporting to clinicians, public health officers and a wider – and ever-increasing – audience. Reporting antimicrobial resistance is considered necessary for selection of empirical therapy based on local data, for assessing the scale of the resistance problem at local, national or international level, for monitoring changes in resistance rates, for detecting the emergence and spread of new resistances, and for providing a measure of the effectiveness of any interventions aimed at reducing resistance. It is essential that results be reported as rapidly as possible to as wide an audience as is thought appropriate, including all those involved in antimicrobial testing, prescribing, supplying and auditing. A proper comparison of data between wards, hospitals or geographical areas (at the local, national and international levels) as well as over time is indispensable for analysing trends and emerging problems.

Treatment of invasive fungal infections

O244 Safety, tolerance and plasma concentrations of voriconazole in immunocompromised paediatric patients

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Objectives: Voriconazole (VCZ) is a novel triazole with broad antifungal spectrum and documented clinical efficacy. Little is known, however, about its use in paediatric patients. We therefore analysed safety, tolerance, and plasma concentrations of VCZ in a cohort of severely immunocompromised patients requiring VCZ therapy.
Methods: The cohort included 37 immunocompromised children and adolescents (2—20 years; mean: 12 years; 12 females and 25 males) with congenital immunodeficiencies (9), AIDS (4), haematological malignancies (21; 14 allogeneic blood stem cell transplant patients), or solid tumours (3) who received VCZ for possible (10)

and probable/proven (14) invasive fungal infections, as primary (4) or secondary (6) prophylaxis or as empirical antifungal therapy (3). Following an intravenous loading dose of 2 × 6mg/kg on day 1, VCZ was administered intravenously and/or orally at dosages ranging from 2 to 8 mg/kg BID until occurrence of intolerance or maximum efficacy.
Results: The 37 patients received VCZ for a mean duration of 174 days (range, 5–998 days). The mean maintenance dosage was 4.31 mg/kg (95% CI, 4.02–4.61 mg/kg). Grade I or II adverse events were observed in 19 patients (51%); the most frequent events included transient increases in hepatic transaminases (19) and transient visual disturbances (5). Four patients (10%) experienced grade III/IV adverse events [reversible increases in serum transaminases (2); reversible increases in serum transaminases and bilirubin (1); increased serum creatinine in a terminally ill patient] and were permanently (3) or transiently (1) discontinued. Mean concentrations of voriconazole in plasma at trough ranged from 0.79 to 3.36 μg/mL without clear dose-dependency ($P = 0.6456$, ANOVA). Twelve of the 14 patients (86%) with probable/proven and all 10 patients with possible infections responded to treatment with VCZ or had stable disease. Twelve of 13 patients who received VCZ as empirical antifungal therapy or for primary or secondary prophylaxis completed therapy without breakthrough infections.
Conclusion: Voriconazole displayed acceptable safety and tolerance and had promising efficacy in treatment and prevention of invasive fungal infections in severely immunocompromised pediatric patients.

O245 Voriconazole compared with a strategy of amphotericin B followed by fluconazole for treatment of candidaemia in non-neutropenic patients

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Objectives: Voriconazole (VOR) is an azole antifungal agent with good *in vitro* activity against most *Candida* spp. We conducted a prospective, randomized, open-label trial comparing voriconazole to a strategy of amphotericin B followed by fluconazole (AMB/FLU) for the primary treatment of candidemia in non-neutropenic patients (pts).
Methods: We enrolled pts with ≥1 positive blood culture (BC) for *Candida* with clinical evidence of infection. Pts were randomly assigned in a 2:1 ratio to receive either VOR (6 mg/kg IV Q12H x2 then 3 mg/kg Q12H) or AMB (0.7–1.0 mg/kg/day for 3–7 days) followed by FLU (400 mg PO QD). All cases were reviewed by an independent, blinded Data Review Committee (DRC). The primary analysis of efficacy compared the proportions of pts surviving with a successful response to treatment, as assessed by the DRC, at 12 weeks after end of treatment (EOT).
Results: Of the 422 pts enrolled, 370 had ≥1 BC for *Candida* within 96 h of entry and were included in the modified intention-to-treat (MITT) analysis. Baseline characteristics were similar in the two treatment groups: the mean APACHE II score was 13.8 for VOR vs. 14.7 for AMB/FLU; the proportion with non-albicans *Candida* was 60.5% for VOR vs. 50.0% for AMB/FLU. BC were still positive at baseline in 304 of 370 (82.2%), and pre-existing indwelling catheters were removed within 3 days of study entry in 281 of 324 (86.6%). The median duration of study treatment was 15 days in both groups: the median duration of AMB was 4 days. In the primary analysis, the proportion with successful outcomes at 12 weeks was 40.72% for VOR and 40.70% for AMB/FLU (difference 0.04, 95% CI: −10.55 to 10.63, non-inferiority margin −15%). In a secondary analysis, which compared the proportions of pts with successful response at the latest relevant timepoint (EOT or 2, 6 or 12 weeks after EOT), the efficacy of VOR was 65.11% compared with 71.33% for AMB/FLU. The median time to negative BC was 2 days in both groups. The Kaplan–Meier survival rates at day 98 were 63.3% for VOR and 57.7% for AMB/FLU (hazard ratio 0.82, 95% CI: 0.58–1.16). The adverse event (AE) profiles were comparable, except for more renal AEs reported in the AMB/FLU group.
Conclusion: Voriconazole is at least as effective as the strategy of amphotericin B followed by fluconazole for the primary treatment of candidaemia, including non-albicans species, in non-neutropenic patients.

O246 Regimens and outcomes of current approaches to secondary prophylaxis after proven or probable invasive fungal infections. A multinational case registry

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Objectives: Allogeneic bone marrow and stem cell transplantation after invasive fungal infection (IFI) has become a feasible option thanks to an expanding arsenal of antifungal agents. Secondary prophylaxis after IFI is being practiced in a number of centres. However, a synopsis of regimens used and of their efficacy is lacking. In this multicentre survey high risk patients with a history of recent proven or probable IFI were followed up for incidence and type of breakthrough IFI during the subsequent allogeneic transplantation.
Methods: We are conducting a survey on SP regimens currently in use in 55 tertiary care centers in 12 countries. Inclusion criteria are: leukaemia/lymphoma, proven/probable IFI according to EORTC/BAMSG during the most recent neutropenic episode with subsequent neutropenia to follow.
Results: Forty-six patients from 19 tertiary care centers in eight European countries were included: prior proven IFI 21 (45.7%; 17 *Aspergillus* spp., two other filamentous, two yeast), prior probable IFI 25 (54.3%; 23 lung, two liver/spleen involvement), 73.9% acute myelogenous leukaemia, median age 38.5 years, range 11–64, 50% female. Patients received secondary prophylaxis with amphotericin B deoxycholate 6.5%, lipid-based 19.6%, fluconazole 4.3%, itraconazole 41.3%, voriconazole 10.9%, caspofungin 10.9% or no prophylactic medication 6.5%. Incidence of breakthrough IFI were 26.1% overall, i.e. 6.5% proven (one *Aspergillus* spp., one *Fusarium* sp.), 8.7% probable (three lung, one liver/spleen), and 10.9% possible (four lung, one liver). Rate of breakthrough IFI by prophylactic antifungal were 1/3 amphotericin B deoxycholate, 1/5 lipid-based, 6/19 itraconazole, 1/5 voriconazole, 0/2 fluconazole, 2/5 caspofungin, and 0/3 without secondary prophylaxis. Overall mortality was 32.6%, of these 33.3% were attributed to IFI.
Conclusion: Incidence of breakthrough secondary IFI and attributable mortality are high in this population undergoing allogenic bone marrow or stem cell transplantation. Controlled studies on secondary prophylaxis are urgently warranted.

O247 Therapy with polyenes and echinocandins in a model of cerebral aspergillosis

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Objectives: Central nervous system (CNS) aspergillosis is the most deadly form of disseminated disease. A recently developed murine model enabled investigation of efficacy of several agents.
Methods: Mice were immunosuppressed and made neutropenic with cyclophosphamide, challenged intracerebrally with *Aspergillus*

fumigatus conidia, and treated daily for 10 days, beginning 1-day post-infection, with AmBisome (AmBi) IV or caspofungin (Cas) or micafungin (Mcf) IP 1, 5, or 10 mg/kg; 1 or 10 mg/kg Abelcet (ABLC) or maximum tolerated dose of 1 mg/kg amphotericin B deoxycholate (AmBd) IV. Survivors were killed on day 14 and residual infection quantitated.
Results: All control mice died. All treatment regimens prolonged survival, with >60% survival from all regimens except Cas 1 and 5 mg/kg and ABLC 1 mg/kg. Mcf 5 mg/kg was optimal and produced significantly prolonged survival over ABLC. No regimen cleared >1 mouse of brain infection. AmBi and ABLC were dose-responsive, whereas the echinocandins were not. Only AmBi 10 mg/kg and ABLC 10 mg/kg significantly ($P < 0.001$) reduced brain infection. In this model, some dissemination to kidney occurs. Only 10 mg/kg AmBi ($P < 0.001$) or ABLC ($P < 0.01$), and Mcf 5 mg/kg and AmBd (both $P < 0.05$) significantly reduced renal CFU, and of 10 mice/group, 4, 1, 0, 1 mice were cleared, respectively.
Conclusion: These data indicate the ability to give more amphotericin in lipid preparations produces an advantage in treating CNS aspergillosis, suggesting a justification for first-line use for that indication, and that the echinocandins prolong survival but do not reduce fungal brain burden.

O248 Posaconazole for *Fusarium* infections: results of two clinical studies

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Objective: *Fusarium* is a genus of ubiquitous fungi that can cause life-threatening disseminated infections in severely compromised hosts. Despite the recent introduction of new antifungal agents, the treatment of these severe disseminated infections remains particularly challenging. We describe the experience in the treatment of these infections with posaconazole, a new oral broad-spectrum triazole antifungal drug.
Methods: Participants in this analysis were patients who had proven or probable infections with *Fusarium* as a primary pathogen and who were enrolled in two phase 2/3 open-label studies of invasive fungal infections. All patients were treated with oral posaconazole suspension 800 mg/day in divided doses. Complete and partial responses at the end of therapy were considered successful outcomes.
Results: Twenty-three patients were included in this analysis; 15 had refractory infections, and eight had non-refractory infections. The patients ranged in age from 8 to 80 years. Most (70%; 16 of 23) had underlying haematologic malignancies, and seven of 16 of these patients had undergone haematopoietic stem cell transplantation. At study entry, 40% (nine of 23) of patients were neutropenic and 17% (four of 23) had graft-vs.-host disease. Most (18) patients were infected with unspeciated *Fusarium*, four had *F. solani*, and one had *F. proliferatum*. The most common clinical presentations were disseminated (11), skin (5), respiratory tract (5), soft tissue (mycetoma; 1), and corneal infection (1). The overall success rates were 48% (11 of 23) for all infections and 36% (four of 11) for disseminated infections. Treatment duration ranged from 1 to 365 days. The success rate was 40% (six of 15) in patients with refractory infections and 62% (five of eight) in patients with non-refractory infections. Posaconazole was well tolerated. The most common adverse events noted were nausea and vomiting.
Conclusions: Oral posaconazole therapy resulted in successful outcomes in 48% of patients, comparable with the success rates for other approved salvage therapies and slightly better than the 30% response rate reported for amphotericin B. This improved success rate was achieved without many of the potential dose-limiting toxicities of other antifungal therapies. Posaconazole is clearly promising as an alternative to therapy with conventional antifungal agents for this often fatal disease.

Sexually transmitted diseases

O249 Performance of the Aptima® CTassay for *Chlamydia trachomatis* and Aptima GC Assay®for *Neisseria gonorrhoeae* using patient- and clinician-collected vaginal swabs

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Objective(s): To assess the performance of Gen-Probe Incorporated's APTIMA CT Assay (ACT) for *Chlamydia trachomatis* (CT) and APTIMA GC Assay (AGC) for *Neisseria gonorrhoeae* (GC) using patient- and clinician-collected vaginal swab (PVS and CVS) specimens. ACT and AGC target rRNA sequences differ from those of the commercially available APTIMA Combo 2 Assay (AC2). The three assays use the same procedures.
Methods: Females are being enrolled at eight geographically diverse, high and low prevalence sites. First catch urine (FCU), one PVS, one CVS, and two endocervical swabs were collected from each subject. ACT and AGC were done on PVS, CVS, FCU, and one endocervical swab. AC2 was done on the same FCU and endocervical swab tested by ACT and AGC. The second endocervical swab and FCU were tested using BDProbeTec (Becton Dickinson and Company) for CT and GC. Subjects were considered infected if two FDA-cleared tests on FCU or endocervical swab were positive.
Results: Of 904 subjects enrolled, results are available for most. There were 97 CT and 50 GC infected subjects (respective prevalences of 12.3 and 6.3%). ACT sensitivities and specificities were 96.9% (94 of 97) and 97.7% (674 of 690) for PVS and 96.9% (94 of 97) and 96.0% (675 of 703) for CVS. AGC sensitivities and specificities were 98.0% (49 of 50) and 99.2% (748 of 754) for PVS and 98.0% (50 of 51) and 99.2% (757 of 763) for CVS. ACT vaginal swab results were in >95% agreement with FCU and endocervical swab results. With AGC, agreement was >98%. Further testing is being performed to determine whether the false positives (FP) are true positives (TP). Previous evaluations of ACT have found that its exquisite sensitivity results in apparent FPs that are shown to be TPs by repeat testing.
Conclusions: These interim findings demonstrate that ACT and AGC using vaginal swabs are sensitive and specific for the detection of CT and GC, respectively.

O250 Molecular techniques in diagnostics of Chlamydiae – benefits and limits

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Objectives: Chlamydiae are important human bacterial pathogens with rather difficult diagnostics because of intracellular part of their growth cycle.

Implementing of molecular techniques provided chance to solve the problem of routine diagnostics. But is it really so?
Methods: Our study performed on nearly 3500 different patients samples compares following diagnostic techniques: culture on

McCoy-cells + microscopy (Iodine and/or Giemsa staining, direct immunofluorescence microscopy (IF Chlamyset), hybridisation-protection assay (GeneProbe) and two versions of PCR-tests (Amplicor and COBAS Amplicor). Additionally, different sample preparation protocols and two different methods for PCR-inhibitors inactivation were compared. Culture and microscopy were used as 'gold-standard'.
Results: IF Chlamyset: Sensitivity 65.41%, Specificity 91.83%, PPV 79.64%, NPV 84.45%; HPA: Sen 79.55%, Sp 96.71%, PPV 92.41%, NPV 90.37%; Amplicor v.1.1: Sen. 85.54%, Sp 96.08%, PPV 91.65%, NPV 92.95%; COBAS Amplicor CT/NG: Sen. 97.63%, Sp. 99.21%, PPV 98.27%, NPV 98.92%. Two freeze-thaw cycles inactivated inhibitors in 77% of samples and harmed DNA in 2% of samples only. With additional cycles dramatically rose damage of target DNA.
Conclusions: PCR test Amplicor CT/NG provided best results vs. gold-standard and may serve as new GS. (Culture vs. new GS Sen.98.27%, Sp. 98.92%, PPV 97.63%, NPV 99.21%.) PCR-inhibitors remain a problem in STD samples. Promising chance for total removal of inhibitors may be hybridisation capture technique for sample preparation.

O251 Clinical relevance of PCR tests for detection of *Treponema pallidum* subsp. pallidum

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Objectives: Syphilis remains a very serious sexually transmitted disease. The increase of incidence in the Czech republic and some other European countries, one-third of which are recent infections including vertical mother to fetus transmission, reminds us its importance. This development returns diagnosis of syphilis back to a daily practice and requires more specific, sensitive and objective methods accessible not only for specialists in venerology, but also for other clinicians. On the contrary a very reliable and user-friendly test is needed. Nucleic acid amplification methods have potential for detection treponemas in blood, cerebrospinal fluid, tissues and lesions.
Methods: Study shows effectivity of tests for different clinical cases and biological materials. It compares specificity and sensitivity of different PCR formats (DIG-labelled nucleotides and anti-DIG ELISA or TaqMan and FRET PCR for LightCycler system) for detection of polA(I) and 47kDa genes of *Treponema pallidum* subsp. pallidum. Results are correlated to a clinical stage and standard direct and indirect tests. T.p. strain Nichols served as positive control, human lymphocytes DNA as negative control.We used Amplicor STD Specimen Collection Kit (Roche) for lesions smears, Amplicor Whole Blood Specimen Preparation Kit (HIV-1QC, Roche) for DNA isolation from smears, whole blood, CSF specimens. An internal control based on co-amplification of beta-globin or beta-actin gene sequences was added to avoid false negative results because of polymerase inhibitors.
Results: Different specimens from 100 patients with clinically and /or serologically verified diagnosis of syphilis were tested. Detection and amplification systems for *polA(I)* gene were more effective than 47 kDa ones. Test sensitivity limit for whole blood was 5×101 T.p.cells/mL (PCR-ELISA format) and 1×101 T.p. cells/mL (LightCycler systems).
Conclusion: PCR TaqMan or FRET probes used in LightCycler were more sensitive than the other ones. The internal control is essential for reliability of test, to avoid false negative results. Method may facilitate diagnosis of syphilis (congenital, neurosyphilis, early syphilis) and also help to recognise infectious risk in a single case. Use in clinical practice demands well experienced laboratory accustomed with clinical counselling.
Acknowledgements: Project supported by grant IGA NI6751-3/2001 (Ministry of Health, Czech Republic).

O252 Laboratory diagnosis of *Trichomonas vaginalis* with culture, ELISA and (real-time) PCR

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Culture of *T. vaginalis* is actually considered the gold standard in laboratory diagnosis. Sensitivity of culture might be negatively influenced by reduced survival of *Trichomonas* during transportation. Diagnosis of Trichomonas by ELISA and PCR techniques do not require the presence of viable parasites. We investigated whether these techniques could provide an alternative for laboratory diagnosis of *T. vaginalis* by culture. 171 clinical samples were cultured and tested by EIA for *Trichomonas vaginalis*. Culture showed four positive samples (2.3%) while *Trichomonas vaginalis* EIA found 12 positive results (7.0%), indicating a tripling of the number of positive results. However, comparison with a conventional PCR for *T. vaginalis* indicated that only five of the samples were true positives. All four culture positive samples were confirmed by PCR while only three of 12 positive samples in the EIA could be confirmed. Using conventional PCR as gold standard, the positive predictive values of culture and EIA were 100 and 27% respectively. A possible explanation for the low predictive value of the EIA was found in further examination of eight EIA positive samples with negative PCR. In seven of eight specimens *Candida albicans* was cultured which might have caused false positive test results because of cross reactivity. The next step was to investigate whether PCR could be a practical alternative for routine diagnosis of *Trichomonas vaginalis*. Conventional PCR was time consuming and labour intensive. A 1-day protocol was developed using automated DNA isolation and real-time PCR. First six culture positive and 39 negative genital swabs showed identical results in real time PCR. Subsequently, 182 urine samples, sent in for laboratory examination for *Chlamydia*, were tested with this real-time PCR protocol. Only two samples showed a positive result. We conclude that the tripling of positive results by EIA compared with culture could not be confirmed with conventional PCR on *T. vaginalis*. Culture has good sensitivity and high positive and negative predictive values. EIA has no additional value compared with culture or PCR. The use of urine as diagnostic specimen for *T. vaginalis* needs further evaluation. Prevalence of *Trichomonas* in our patient population remains low and therefore there is a need for reliable sensitive diagnostic tools. Real time PCR has been made available as a potentially useful alternative for routine diagnosis of *Trichomonas vaginalis*.

O253 Efficacy of long-term suppressive therapy with valacyclovir in recurrent genital herpes

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Objectives: Recurrent genital herpes (RGH) has several treatment options. One of them is chronic suppressive therapy with antiviral compounds. Present study was performed to assess the efficacy and safety of long-term valacyclovir for RGH.
Methods: During 1999–2003 in Smolensk 42 patients of both sexes aged 18–55 years with the history of RGH (duration 1–20 years; four to nine recurrences/year) were in double-blind manner randomly assigned for valacyclovir 500 mg (22 patients) or placebo (20 patients) daily for 8 months. Thereafter all of them were given open-label valacyclovir at the above dose for another 12 months. Only HSV2+ve / HSV1+/–ve patients (serostatus was determined with Western-Blot technique) were eligible for the study. During the double-blind phase every recurrence was treated with valacyclovir 500 mg twice daily for 5 days. On the contrary, none of the patients received episodic treatment but chronic suppressive therapy during open-label phase.
Results: During the double-blind phase statistically significant recurrences frequency reduction in comparison with disease history was registered in both treatment arms ($P < 0.05$). However, while analysing individual cases this was observed in all

'valacyclovir' group patients but only in 75% of 'placebo' group. Similarly, absence of clinical episodes of GH was documented in 68.2 and 40% of patients, respectively. Patients from 'placebo' group experienced significantly more frequent recurrences than ones from 'valacyclovir' group ($P < 0.05$). Overall recurrences rate in former group was 4.8 times higher comparing to active treatment. Further continuation of suppressive therapy up to 20 months has not given any advantages to 8-month treatment in terms of decreasing GH recurrence rate. The nature and frequency of adverse events for valacyclovir and placebo were similar.
Conclusion: Daily suppressive therapy with valacyclovir 500 mg is a highly effective method for prophylaxis of recurrent genital herpes though it does not eradicate all recurrences. Prolongation of such therapy from 8 to 20 months does not decrease recurrence rate any further.

Pharmacokinetics and pharmacodynamics

O254 Plasma and intrapulmonary concentrations of oritavancin and vancomycin in normal healthy adults

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Background: Oritavancin is a semisynthetic glycopeptide with bactericidal activity against Gram-positive pathogens, including resistant pulmonary pathogens such as methicillin-resistant *Staphylococcus aureus* and drug-resistant *Streptococcus pneumoniae*.
Objective: We compared the concentrations of oritavancin and vancomycin in plasma (P), epithelial lining fluid (ELF) and alveolar macrophages (AM) in adults who underwent bronchoscopy and bronchoalveolar lavage (BAL).
Methods: Thirty nonsmoking adults (age: 20–54 years; 12 males, 18 females) were randomised to receive intravenous oritavancin 800 mg once-daily for five doses ($n = 20$) or vancomycin 1000 mg q12h for nine doses ($n = 10$). Serial P drug samples were collected up to 504 (oritavancin) and 24 h (vancomycin) after the last dose. Each subject had a single bronchoscopy and BAL following the last dose at 4, 12, 24 or 168 h (oritavancin) and 4 or 12 h (vancomycin) (five subjects per sampling time).
Results: Mean ± SD concentrations (μg/mL) in P, ELF and AM are presented in the Table. Both drugs (oritavancin vs. vancomycin) displayed multiexponential decline in P with a mean Cmax of 183.7 vs. 49.4 μg/mL and AUC0–24 of 2310 vs. 367 μg·h/mL. Using mean intrapulmonary concentrations, the AUC0–24 in ELF were 106 vs. 92 μg·h/mL and 3297 vs. 926 μg·h/mL in AM.

	4-h	12-h	24-h	168-h
P Oritavancin	119.6 ± 24.6	75.7 ± 16.3	73.7 ± 28.2	10.4 ± 3.0
ELF Oritavancin	3.1 ± 1.1	3.7 ± 2.5	6.3 ± 1.5	1.7 ± 0.8
AM Oritavancin	121.0 ± 69.1	113.1 ± 49.7	179.4 ± 76.7	557.9 ± 481.6
P Vancomycin	19.8 ± 3.7	5.1 ± 1.7		
ELF Vancomycin	5.3 ± 1.5	2.4 ± 0.7		
AM Vancomycin	32.0 ± 8.5	45.2 ± 23.3		

Conclusions: Oritavancin is slowly distributed into and out of the ELF and AM. The ELF concentrations of oritavancin and vancomycin were similar in magnitude. Oritavancin was more concentrated in macrophages of the lung compared with vancomycin. The intrapulmonary concentrations lend support to the potential effectiveness of oritavancin 800 mg once-daily for the treatment of susceptible Gram-positive pathogens associated with lower respiratory tract infections.

O255 Should we routinely monitor β-lactam serum concentrations during the treatment of endocarditis?

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Objectives: Because of pharmacokinetics and pharmacodynamics concerns, high dosages of β-Lactam (BL) are used for the treatment of endocarditis. Guidelines do not take into account many factors related to the host, and they have not significantly changed during the last four decades although the population of patients with endocarditis has dramatically changed with a mean age of 35 years in 1940 and 60 years in 2000. BL are considered as drugs with few dose-related side-effects. However, very high serum concentrations could be toxic on central nervous system, especially in elderly patients. The aim of this study was to evaluate the range of BL trough serum concentrations obtained when the guidelines on the treatment of endocarditis are applied.
Methods: We performed a retrospective study of all patients with documented bacterial endocarditis-treated according to the guidelines for whom BL trough serum concentrations were measured at the request of the physician because of tolerance or efficacy concerns in our department from 2000 to 2002. The concentrations were determined by high-performance liquid chromatography (HPLC).
Results: Fifteen patients were studied (12 males). Mean age was 62 years (22–79). The bacteria isolated in blood cultures included organisms of the genera *Streptococcus* ($n = 5$), *Enterococcus fecalis* ($n = 5$), *Staphylococcus aureus* ($n = 4$) and *Klebsiella oxytoca* ($n = 1$). MIC of the BL used was determined in 10 cases and was always between 0.05 and 0.4 mg/L. Amoxicillin trough serum concentrations were determined 11 times, in nine patients. The mean value was 86.8 mg/L (range: 30–212), and trough concentrations were above 1000 times the MIC in five cases. Cloxacillin trough serum concentrations were determined in three patients. The mean value was 47.9 mg/L (range: 16.7–104). For nine patients, BL serum concentrations were measured because dose-related intolerance was suspected, including renal failure ($n = 8$), and/or confusion ($n = 3$): In seven of these patients, BL trough serum concentrations were >40 mg/L and led to a reduction in BL dosage with a subsequent improvement of renal function and/or mental status.
Conclusion: Following the guidelines for the treatment of endocarditis, we observed high and unpredicted values of trough serum concentrations of BL. The development of HPLC could offer an opportunity to monitor BL serum concentrations and would allow individualised rather than standardised treatment for endocarditis.

O256 Utility of the 750 mg dose of levofloxacin against *Streptococcus pneumoniae* as assessed by Monte Carlo simulation and expectation over a large MIC distribution

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Objectives: Levofloxacin has recently been approved for short course therapy of community-acquired pneumonia at a dose of 750 mg. Given the results of a recent surveillance study of 4452 strains of *Streptococcus pneumoniae* (TRUST VII data set), we wished to determine how well the 750 mg dose attained the target-free drug AUC/MIC ratio of 30, associated with a good microbiological outcome, as described by Ambrose.
Methods: The population pharmacokinetic parameters derived from a study of 272 patients treated with levofloxacin for

community-acquired infections were used to generate a 10 000 subject Monte Carlo simulation. The ADAPT II package of programmes of D'Argenio and Schumitzky was employed for generation of this Monte Carlo simulation. Both normal and log-normal distributions were evaluated. An expectation was taken over the entire distribution of pneumococcal levofloxacin MICs from TRUST VII to generate a population target attainment rate accounting for both pharmacokinetic and MIC variability.
Results: The 4452 strains of *S. pneumoniae* had levofloxacin MIC values distributed such that 1% were <0.25 mg/L; 32.3% were at 0.5 mg/L; 64.7% were at 1.0 mg/L; 0.7% were at 2.0 mg/L; 1% were >2.0 mg/L. The log-normal distribution recreated the parameter values and dispersions with greater fidelity and was employed. The target attainment rate for a free drug AUC/MIC ratio of 30 was 95.2% at 1.0 mg/L. Other, lower MICs were >99.9% in target attainment. The population target attainment, accounting for the variability in pharmacokinetics (10 000 subject Monte Carlo simulation) and variability in MIC for *S. pneumoniae* (4452 strains) was 95.6%.
Conclusion: The 750 mg dose of levofloxacin provides an excellent population target attainment rate, providing a high probability (95.6%) of developing a free drug AUC/MIC ratio of 30, concordant with the clinical and microbiological effects seen in clinical trials.

O257 Use of pharmacodynamics and Monte Carlo simulations to predict clinical cure of *Pseudomonas aeruginosa* infection

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Dijon, F

Objectives: Pharmacodynamics (PD) indices such as Peak/MIC (QImax), time above MIC (T > MIC), and AUC24/MIC have been linked to clinical outcome for numerous antibiotics. The use of pharmacokinetic (PK) models allows optimising both the dose and interval of drug at its MIC to obtain the threshold value of the PD indices. Our objective was to evaluate the frequency with which the major antibiotics used to treat *Pseudomonas aeruginosa* infections attained PD index targets.
Methods: Three hundred and nine strains of *P. aeruginosa* were collected from patients hospitalised in 2003. MICs were determined according to CA-SFM standards. Plasma concentrations were calculated using a compartmental PK model based on published PK data. They were adapted on renal function of each patient. MICs distribution (susceptible strains) and patient-specific data including calculated creatinine clearance (Cockroft), age, gender and body weight were used to conduct Monte Carlo simulations of 5000 patients. The evaluated treatments were ceftazidime (CAZ; 1 g tid, 2 g tid, 4 g continuous infusion), imipenem (IMP; 1 g tid), piperacillin + tazobactam (TZP; 4 g tid), amikacin (AKN; 15 mg/kg od), and ciprofloxacin (CIP; 400 mg IV tid). The forecasts were focused on a $Qi_{max} > 10$ for aminoglycosides and fluoroquinolones, T > CMI > 70% for beta-lactams, and AUC24/MIC > 125 for all classes of antibiotics.
Results: A 6% of the strains were I/R for AKN, 7% for TZP, 14% for CAZ, 31% for IPM and 27% for CIP. The values of the PK parameters obtained with the model we used were identical to those reported in the literature. The frequency with which the fixed threshold was attained exceeded 95% for beta-lactams. It was 73% for CIP, and the percentage fell to 41% with AKN, even when an individualised and adapted administration's interval was used. Frequencies of PD success with antibiotics schemes evaluated were <5% when testing *P. aeruginosa* I/R strains, except with CAZ 2 g tid (23%) or 4 g continuous infusion (42%).
Conclusions: When using Monte Carlo simulation modelling, beta-lactams generate high frequencies of PD success on infections caused by *P. aeruginosa* susceptible strains and sometimes by I/R strains when using CAZ. Similar PD success is not available for AKN or CIP. These results can explain the high frequency of *in vivo* resistant mutants and suggest that higher doses and/or combination therapy may be necessary to achieve optimal PD indices when using aminoglycosides or fluoroquinolones.

O258 Development of a pharmacokinetic/pharmacodynamic model to characterise the *in vitro* activity and dose–response relationships of daptomycin and linezolid against vancomycin-resistant enterococci (VRE) using subpopulation analysis

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Background: *In vitro* and animal PK/PD infection models are predictive of drug response in patients. Our purpose was to develop a mathematical PK/PD model for time-kill experiments, accounting for bacterial subpopulations, which could be adapted to *in vitro* and animal PK/PD infection models to improve translational PK/PD research between these experimental systems.
Methods: Time-kill experiments were performed according to NCCLS standards against six clinical bloodstream VRE isolates with LZD and DAP at concentrations of 0, 0.5, 1, 2, 5 and 10x MIC. Bacterial counts (CFU/mL) determined at 0, 1, 2, 4, 6 and 24 h. Candidate PK/PD models were fit to the time course of bacterial counts (ML, ADAPT II); model discrimination by Akaike's Information Criterion. Final PK/PD parameters were determined by MAP-Bayesian estimation.
Results: DAP demonstrated bactericidal activity against all isolates (>3 log kill at 24 h), while LZD was bacteriostatic. A mixture model of three bacterial subpopulations with varying drug susceptibilities best fit the data for both drugs. The final PK/PD model includes a capacity-limited bacterial growth function (parameters Vg_{max}, CFU_m: the maximum bacterial growth rate and number of bacteria at which growth is half-maximal), and 1st-order bacterial death rate constant (K_d); and drug may either enhance bacterial kill or inhibit growth through a Hill-type function (H, Hill's constant; SIT_{ms}, SIT_{mi}, SIT_{mr} are the relative concentrations of drug where drug effect is half-maximal for the most 'sensitive', 'intermediate', and most 'resistant' subpopulations, respectively). The relative % of the total bacterial inoculum for each subpopulation (%S, %I, %R) and each organism was determined by model fitting of the initial conditions. The fit of the model to the data was excellent, with an overall $r^2 = 0.96$. A summary of PK/PD parameters (mean, CV%) for all organisms is presented in the table below.

	Vgmax (CFU/h)	CFUm (CFU)	Kd (1/h)	H	SITms (mg/L)	SITmi (mg/L)	SITmr (mg/L)	%S (%)	%I (%)	%R (%)
DAP	2.6×10^8(98)	8.1×10^7(56)	0.21(22)	2.0(9.8)	0.37(11)	0.74(7.6)	3.8(16)	3.5(34)	95.2(14)	0.7(116)
LZD	9.6×10^7(39)	6.7×10^7(68)	0.16(14)	2.1(4.2)	0.46(17)	1.02(35)	3.7(16)	0.001(24)	99.7(0.001)	0.28(12)

Conclusions: Daptomycin demonstrated significantly greater kill against clinical isolates of VRE compared with linezolid, and the model predicted more variability in subpopulations. The time courses of kill curve experiments for both bactericidal and bacteriostatic antibiotics were well characterised by a PK/PD model incorporating bacterial subpopulations of varying drug susceptibilities. This approach to PK/PD modelling of static *in vitro* data of a concentration range over time may aid in translating results to dynamic *in vitro* and animal PK/PD infection models.

Group A streptococcal disease in the 21st century

S263 Strep-EURO project: progress report

A. Jasir – Strep-EURO Study Group

To improve understanding of the epidemiology of severe group A streptococcal (GAS) disease in Europe and achieve an integrated picture of these infections, Strep-EURO project started September 2002. A European case definition for severe invasive GAS disease was agreed upon amongst all Strep-EURO participating countries. The definition of the 1993 Working group recommendations for classification of streptococcal infections was used in order to trace patients with severe invasive infections and to obtain the corresponding GAS isolates. In order to obtain nationwide coverage, information on the project was given to infectious control units, relevant hospitals and microbiological laboratories. A clinical questionnaire containing background identification data, possible predisposing factors, portal of entry of infection, epidemiological risk factors, source of isolate, clinical condition, complications and outcome, was used for reporting of each case. Questionnaires were translated in each respective country. Enhanced surveillance of GAS invasive disease commenced in all participating countries from 1 January 2003 for a 2-year period. A total of 1753 invasive GAS isolates were collected during the first 6 months. These strains were subjected to various investigations. Molecular methods for typing and clonal identification of clinical isolates were used. Strains were also analysed for antibiotic susceptibility, and resistance determinants. Furthermore, pathogenic aspects of severe GAS disease, such as proteolytic activation of the contact system, and selected virulence factors, such as surface proteins and superantigens, were investigated. A new Strep-EURO website was constructed and updated regularly. The website is linked with other, relevant websites and contains both an 'intra-net' site accessible to those persons participating within the project, and an 'extra-net' site for general public. A database for severe GAS infections was established. Clinical and laboratory information related to each case will be registered from the questionnaire into local databases, which have been constructed in a standardised way in order to be conveniently entered into the main database. The database files are available on the Strep-EURO website. Strep-EURO has now created a European network for epidemiological analysis and surveillance of severe streptococcal disease in 11 EU and Associated Countries.

S264 Severe group A streptococcal infections in Europe: microbiological and epidemiological aspects

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Streptococcus pyogenes (Lancefield group A streptococcus, GAS) causes a wide range of invasive diseases, including bacteraemia, necrotising fasciitis, streptococcal toxic shock syndrome (STSS) and pneumonia. In spring 1994, a cluster of cases of necrotising fasciitis in an area of England attracted global media interest. Many countries, particularly in Europe, reassessed their surveillance strategies for severe GAS infections. Enhanced surveillance of invasive GAS disease was carried out in England and Wales from mid-1994 to mid-1997. The United Kingdom Reference Laboratory received isolates from 1913 cases. Rates of disease varied with age from 0.84 per 100 000 populations in males and 1.0 in females aged 0–10 years to 7.7 in males and 5.1 in females aged over 80 years. The median age of all cases was 57 years and of fatal cases was 71 years. The overall mortality rate was 27%, and the highest rates occurred in the oldest age groups. Most cases presented with bacteraemia (76%); other presentations included STSS (9%), septic arthritis (8%), necrotising fasciitis (6%) and pneumonia (5%). One-fifth of cases were admitted to intensive therapy units. The most commonly reported predisposing factors were skin lesions (38%), a third of which were reported to be due to trauma, and 11% of cases were reported to be immunocompromised. A total of 13 clusters (two or more linked cases of invasive GAS infection) were identified. The predominant GAS serotypes were M1 (30% of cases and 41% of deaths), M3 (10% and 14%) and type 28 (10 and 7%). The second period of enhanced surveillance was established under the auspices of the strep-EURO programme from January 2002 for 2 years. Preliminary data suggest that the incidence of severe disease in the UK is considerably higher than previously reported. In 2002, more than 1500 cases of invasive GAS disease were reported in the UK; an overall incidence of 2.0 per 100 000 populations. The overall mortality rate and the distribution of clinical presentations was similar to those observed in previously. There were differences in the distribution of serotypes, with the emergence of higher M types.

S265 Multilocus sequence typing of group A streptococci in invasive infections

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The WHO Collaborating Center for Reference and Research on Streptococci in Prague participates in the EU project Strep-EURO. One of the working packages includes implementation of active surveillance of severe infections caused by group A streptococci (GAS) and investigation of GAS isolates. All strains isolated within this surveillance are typed in our laboratory by classical methods and a sequencing method, emm typing. In addition, we have decided to use multiloci sequence typing (MLST) for clonal analysis of GAS isolated in active surveillance in the first 6 months of the year 2003. DNA was extracted as described at emm website (http://www.cdc.gov/ncidod/biotech/strep/protocols.htm). Internal fragments of seven housekeeping genes (*gki, gtr, murI, mutS, recP, xpt, yqiL*) were amplified. Purification of DNA fragments was done using enzyme ExoSAP-IT (Amersham-Pharmacia). Sequencing on both strands of each gene was performed and final trimmed sequences were submitted to the MLST-strep database (http://spyogenes.mlst.net) to obtain allelic profile and to assign a sequence type (ST). Our invasive GAS (29 isolates) mostly belonged to emm type 1, followed by emm66, emm81 and surprisingly emm108. Some of the emm 1 isolates were identified as ST 28. At the time of writing this abstract, MLST was not yet finished for all isolates? however, we have found the same correlation between emm types and STs as described by other authors. We believe that MLST is very useful for the investigation of clonality of GAS isolates. Further study of isolates from severe infections, carriers and/or pharyngitis would allow identification of hypervirulent complexes using MLST.

This study was supported by research grant EU Strep-EURO Contract no. QLRT-2001-01398 and made use of the Multi Locus Sequence Typing website (http://spyogenes.mlst.net).

S266 Pathogenic aspects on severe group A streptococcal infections

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Severe infections caused by group A streptococci (GAS) include a toxic shock syndrome (TSS) and necrotising fasciitis (NF), both implying high mortality rates, but also less fulminant disease such as septicaemia, erysipelas, cellulitis, endometritis and pneumonia. The majority of GAS infections, like pharyngotonsillitis and impetigo, however, are superficially located. Virulence properties specific for invasive GAS strains have not been identified but a variety of new mechanisms of possible role for acute manifestations have been characterised. As well recognised, massive activa-

tion of T cells by GAS superantigens, with release of proinflammatory cytokines, may elicit severe clinical symptoms. Activation of the contact and complement systems by GAS may be of great significance by induction of shock and tissue damage. Cleavage of plasminogen by streptokinase to proteolytically active plasmin may play an important role in soft tissue infections. Out of more than 150 known M-types, only a minority, such as types 1, 3, 12 and 28, seem to be regularly involved in TSS and NF, indicating a crucial role in this context of M protein or other type-variable factors. Together with the hyaluronate capsule, the M proteins are believed to promote evasion of phagocytosis, allowing for rapid growth of GAS in fresh human blood. Interactions of M proteins with plasma proteins are of special interest, as deposition of opsonic C3b on the bacterial surface seems to be prevented by the binding to GAS of complement regulators C4BP and factor H (FHL-1) and as well fibrinogen. The nonimmune binding of IgA Fc by GAS, occurring in many strains, may serve to block ingestion of the bacteria through phagocytic IgA receptors. Furthermore, direct binding of IgG Fc to the bacteria can interfere with antibody-mediated opsonisation of GAS, as may also specific cleavage of the IgG molecule by some GAS enzymes. Cleavage of chemotactic C5a, shown by all GAS strains, is another example of interference of GAS with host defence systems. In contrast to increased knowledge about immediate mechanisms, the pathogenic events leading to the major nonsuppurative complications of GAS disease, acute rheumatic fever and poststreptococcal glomerulonephritis, remain elusive. However, enhanced understanding of virulence mechanisms is now providing a broadened basis for design of immunoprophylaxis and new therapies against this important pathogen.

Susceptibility testing and its role in therapy and epidemiology (Symposium arranged with EUCAST)

S273 Antimicrobial resistance frequencies using EUCAST epidemiological cut-off values compared with national breakpoints when applied to the EARSS database

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Objectives: European Committee on Antimicrobial Susceptibility Testing (EUCAST) is harmonising European breakpoints and defining epidemiological cut-off values (*JAC* **52**: 145–148, 2003). European national breakpoint committees in (BSAC, CA-SFM, CRG, DIN, NWGA, SRGA) have determined breakpoints for decades. To complicate the situation further, most European laboratories use recommendations from NCCLS (USA), and breakpoints often differ quite substantially between guidelines. Nevertheless they are used for both clinical and surveillance purposes. This talk presents the theoretical impact on resistance rates of using the different guidelines for surveillance.
Methods: Of the total number of routinely collected antibiotic susceptibility test (AST) results for invasive *S. aureus*, *S. pneumoniae*, and *E. coli* in the EARSS database ($n = 103\,472$), the available MICs ($n = 18\,938$) for different drug–bug combinations were reinterpreted according to the different national guidelines and the EUCAST epidemiological cut-off values.
Results: In a number of instances the world is already agreed on how to differentiate between susceptible and resistant bacteria; MRSA, penicillin-nonsusceptible *S. pneumoniae*; vancomycin resistance in *Enterococcus* species. In other instances, variations may be substantial but resistance is uncommon or clear-cut with unequivocal changes in MIC. However, in some instances the quantitation of resistance is severely affected by the choice of breakpoint – the range of resistance frequencies obtained with the breakpoints from the various committees are given and in parenthesis the rate obtained with the tentative EUCAST epidemiological cut-off value (ECOFF); *E. coli* vs. gentamicin 1.9–14.3% (ECOFF: 5.5%), vs. cefotaxime 0.6–2.6% (ECOFF 5%), vs. ciprofloxacin 3.9–8.3% (ECOFF 12.7%); *S. pneumoniae* vs. erythromycin 16–24.1% (ECOFF 24.1%); *S. aureus* vs. ciprofloxacin 7.2–12.4% (ECOFF 12.4%) and vs. erythromycin 2.9–8.3% (ECOFF 8.3%).
Conclusions: Many breakpoint differences had marginal effects on epidemiological resistance rate estimates. In other instances the choice of breakpoint affected the rate substantially. The discrepancies encourage a European harmonisation of breakpoints and the introduction of common epidemiological cut-off values for the comparison of resistance rates.

Antibiotic resistance mechanisms

O275 Diversity and evolution of class A chromosomal beta-lactamase genes in *Klebsiella pneumoniae*

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Objectives: To investigate the diversity of the class A chromosomal beta-lactamase gene (*bla*) in *Klebsiella pneumoniae*, in order to study the evolution of this gene, and to determine whether *bla* co-evolves with housekeeping genes.
Methods: Nucleotide sequencing of *bla* and the housekeeping genes *gyrA*, coding for subunit A of gyrase, and *mdh*, coding for malate dehydrogenase, was performed on a panel of 28 *K. pneumoniae* strains. The strains were representatives (i) of three phylogenetic groups, KpI, KpII and KpIII, recently identified, and (ii) having different chromosomal beta-lactamase variants previously identified. Isoelectric focusing of the beta-lactamases was performed on cell-free sonicates. Susceptibility testing was performed using *E*-tests.
Results: Three groups of *bla* sequences were found; two of them included variants of SHV (pI 7.6) and LEN (pI 7.1), respectively. One new SHV variant and seven new LEN variants were identified. The third group, more heterogeneous, included four other *bla* variants. These variants formed two subgroups, one with pIs 7.8 and 8.1, and the other with pIs 6.5 and 7.0. Susceptibility to ampicillin, cefuroxime, cefotaxime, ceftazidime and aztreonam, and inhibition by clavulanic acid were similar in the three

groups. The phylogenies based on *bla*, *gyrA* and *mdh* sequences revealed a parallel evolution for the three genes. The group comprising SHV-type *bla* sequences included all strains belonging to the phylogenetic group KpI. The LEN-type *bla* sequences were all from KpIII strains. The third group comprised *bla* sequences from all KpII strains. The correspondence between *bla* sequences and phylogenetic groups was fully confirmed on 34 additional strains in PCR assays that were developed to be specific for the three *bla* groups. Based on the levels of synonymous substitution, we estimated the time since divergence of the *K. pneumoniae* phylogenetic groups to range between 6 and 28 million years.

Conclusion: Our results demonstrate a high diversity of the chromosomal class A beta-lactamase gene in *K. pneumoniae*, and a coevolution of this gene and the housekeeping genes *gyrA* and *mdh*. In addition, they show that this beta-lactamase gene was anciently present in the genome of *K. pneumoniae*, and that the beta-lactamases SHV and LEN correspond to the native beta-lactamases of phylogenetic groups KpI and KpIII, respectively.

O276 Resistance to beta-lactam antibiotics in *Staphylococcus aureus*

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Objective: Emergence of bacterial strains designated as methicillin-resistant *Staphylococcus aureus* (MRSA) from 1960s to the present has created a state of crisis for nosocominal infections worldwide. The gene product of *mecA* is a penicillin-binding protein (PBP) designated PBP2a. *Staphylococcus aureus* normally produces four PBPs, enzymes that are anchored on the bacterial cytoplasmic membrane, whose functions are the assembly and regulation of the latter stages of the cell wall biosynthesis. Whereas these four PBPs are susceptible to modification by β-lactam antibiotics, an event that leads to bacterial death, PBP2a is refractory to the action of all β-lactam antibiotics. Furthermore, PBP2a is capable of taking over the functions of the four typical PBPs of *S. aureus* in the face of the challenge by β-lactam antibiotics. We studied the basis for resistance of this protein to inhibition by β-lactam antibiotics.

Methods: The *mecA* gene was cloned and expressed in *E. coli*, and PBP2a was purified to homogeneity for biochemical analyses.

Results: The kinetic parameters for interactions of several β-lactam antibiotics (penicillins, cephalosporins and a carbapenem) and PBP2a were evaluated. The enzyme manifests resistance to modification by β-lactam antibiotics at the active-site serine residue in two ways. First, the microscopic rate constant for acylation (k2) is attenuated by 3–4 orders of magnitude over the corresponding determinations for penicillin-sensitive PBPs. Secondly, the enzyme shows elevated dissociation constants (K_d) for the preacylation complexes with the antibiotics, the formation of which ultimately would lead to enzyme acylation. The two factors working in concert effectively prevent enzyme modification by the antibiotics *in vivo*, giving rise to drug resistance. The enzyme undergoes a dramatic conformational change in the course of these events in *in vitro* experiments, which should be critical for the biological function of the protein.

Conclusions: The recent emergence of variants of MRSA resistant to oxazolidinone and glycopeptide antibiotics has created a situation that certain strains of *S. aureus* are either treatable only with a single class of antibiotics or are simply not treatable, which is a disconcerting situation clinically. These studies provide the first mechanistic details for this critical enzyme, presenting opportunities in antibiotic design.

O277 Analysis of the flexible loop of the SPM-1 metallo-beta-lactamase in substrate binding and hydrolysis

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Objectives: Molecular modelling of SPM-1 showed that it has a very similar structure to IMP-1, differing primarily in the flexible 24 amino acid loop unique to SPM-1. SPM-1 and IMP-1 show very different kinetics, indicating a hydrolytic role for the loop. Accordingly, the loop was deleted and substrate binding and hydrolysis and zinc content were compared with wild-type SPM-1 (wtSPM-1).

Methods: The 24 amino acid loop was deleted from SPM-1 (SPM-Ldel) by PCR overlapping primer extension techniques and confirmed by sequencing. The PCR product was over-expressed in *Escherichia coli* and confirmed by PCR using SPM-1-specific primers. RT-PCR was used to verify gene expression. SPM-Ldel was purified using an ammonium sulphate precipitation and standard protein chromatography, and the hydrolytic profile was determined for, cefuroxime, penicillin G, nitrocefin, imipenem and meropenem. Zinc assays were undertaken using Unicam 919 atomic absorption spectroscopy.

Results: Sequence analysis verified the deletion and RT-PCR confirmed gene expression. The SPM-Ldel gave kcats of 0.07 s^{-1} and 0.14 s^{-1} for imipenem and meropenem, respectively, compared with 33 and 63 s^{-1}, for the wtSPM-1. K_m values decreased from 281 to 61 μM for meropenem and from 37 to 9.5 μM for imipenem. For cefuroxime, k_{cat} and K_m values changed from 37 to 0.6 s^{-1} and from 4 to 5 μM, respectively. SPM-Ldel weakly hydrolysed penicillin with a kcat of 3.17 s^{-1} and a K_m of 226 compared with the wtSPM-1 (k_{cat} 108 s^{-1} and K_m of 38 μM). Interestingly, the nitrocefin k_{cat} value did not markedly change (0.53 s^{-1} for wtSPM-1 compared with 0.51 s^{-1} for SPM-Ldel) and the K_m increased from 4 to 23 μM.

Conclusions: Unexpectedly, removal of the loop did not consistently result in weaker substrate binding. The lower kcat values for SPM-Ldel demonstrate that the loop is integral to the hydrolysis of beta-lactams by SPM-1.

O278 Hospital outbreak of an imipenem-resistant VIM-2 encoding *Acinetobacter* DNA group 14 TU strain in a German teaching hospital

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Objective: To characterise the resistance mechanism of a carbapenem-resistant haemolytic *Acinetobacter* strain which was recovered from cultures of blood of three patients with bloodstream infection following coronary angiography. A carbapenem-sensitive strain was recovered from a fourth patient involved in the same outbreak.

Methods: Species identification of *Acinetobacter* isolates was performed by amplified ribosomal DNA restriction analysis (ARDRA). Strain relatedness was assessed by pulsed-field gel electrophoresis (PFGE). Multi-resistant *Acinetobacter* strains were initially analysed with Etest MBL strips and positive strains were further investigated by PCR using class 1 integron primers designed to the conserved 5′ and 3′ sequences. Sequencing of PCR amplicons was undertaken on both strands by the dideoxy-chain termination method. Sequence analysis was performed using the Lasergene DNASTAR software package.

Results: All four strains were identified as unnamed *Acinetobacter* DNA group 14 TU. The three carbapenem-resistant isolates exhibited an identical PFGE pattern whereas the carbapenem-sensitive strain showed a single band shift, the four isolates thus represented a single clone. The carbapenem-resistant isolates tested positive for production of a metallo-beta-lactamase (MBL) using Etest MBL strips with a reduction of MIC from 64 to 3 mg/L in the presence of EDTA. Crude cell extracts demonstrated moderate hydrolytic activity against carbapenem antibiotics that was inhibited by EDTA confirming the phenotypic expression of an MBL. Amplification and sequencing identified a class1 integron containing two resistance gene cassettes with the blaVIM-2 MBL gene cassette in the first position followed by the *aacA4* gene. The sequence of the integron indicated that it is a class 1 type and embedded in a Tn21-like mobile genetic element. The combination of both genetic elements make would make these cassettes highly mobile although, to date, transfer to other bacteria could not be demonstrated.

Conclusions: This is the first report of a hospital outbreak caused by *Acinetobacter* DNA group 14 and the first report of carbapenem resistance in *Acinetobacter* species other than *A. baumannii*. It is also the first report of VIM-2 in Germany and the second report of a class1 integron containing a *MBL* gene in this nation (along with GIM-1). The emergence of these *MBL* genes in clinical pathogens is a serious challenge to current chemotherapy.

O279 Defining the meropenem susceptible population in *Pseudomonas aeruginosa* by analysing expression of resistance genes in clinical isolates with different levels of meropenem resistance

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Objectives: Analysis of meropenem disk-diffusion zone distributions for *Pseudomonas aeruginosa* at Karolinska University Hospital revealed a unimodal pattern without a distinct border between the susceptible and resistant population, similar to that reported earlier by the British Society for Antimicrobial Chemotherapy (BSAC). However, the Swedish Reference Group for antibiotics has, in contrast to BSAC, chosen to define also an intermediary susceptible category. Furthermore, Swedish MIC breakpoint for meropenem susceptibility is $\leq$2 mg/L, compared with the BSAC/NCCLS breakpoint at 4 mg/L. The rationale for these differences is that isolates on the border between susceptible and resistant are believed to consist mainly of OprD-negative mutants that have slight increases of meropenem MIC-values compared with wild-type bacteria, but are still considered meropenem susceptible. In this study we examined the expression of meropenem resistance genes in clinical isolates, to be able to provide molecular evidence for defining differences between the populations.
Methods: Twenty clinical isolates of *P. aeruginosa* with different levels of meropenem resistance were examined with real-time reverse transcriptase PCR for expression of resistance genes *oprD*, *mexB* and *mexD*. Strain ATCC 27853 and three other isolates were meropenem susceptible, four were borderline susceptible, four were intermediary susceptible, four were borderline resistant and four were resistant. Three of the meropenem intermediary susceptible isolates were also imipenem resistant. Meropenem MIC was determined for all isolates.
Results: The meropenem intermediary susceptible group was heterogenous regarding gene expression. Two imipenem-resistant isolates with meropenem MICs of 2 and 4 mg/L had only decreased expression of *oprD*. The imipenem susceptible isolate had a meropenem MIC of 4 mg/L and a significant increase of *mexB*, while *oprD* and *mexD* expression was comparable to the ATCC strain. The third imipenem-resistant isolate had significantly decreased *oprD* level, as well as significant increase in expression of both efflux genes.
Conclusions: Our findings indicate that the Swedish intermediary susceptible population, mostly isolates with MICs of 4 mg/L, does not only consist of OprD-negative mutants, but also of isolates with overexpression of efflux genes. We believe that this should be taken into consideration when defining the meropenem susceptible population.

O280 Overexpression of Rob increases MIC to norfloxacin by upregulation of the MdfA efflux pump in *Escherichia coli in vitro*

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Objective: In *E. coli* various efflux pump systems like AcrAB, AcrEF, EmrAB and MdfA are known to confer resistance to various classes of antimicrobials. Several transcriptional factors like MarA, Rob and SoxS are involved in their regulation. Here we report how overexpression of Rob affected the transcription of the acrAB, mdfA, emrAB and norE efflux pump systems, all known to confer resistance to fluoroquinolones.
Methods: Rob was overexpressed from the arabinose-inducible plasmid pCC1 (Epicentre®) under control of the lac promoter in *E. coli* (pCC1-lacrob). MICs (Etest®, Solna, Sveden) to norfloxacin (NFX) and ciprofloxacin (CIP) and transcription levels of gapA, Rob, mdfA, acrAB, acrD, acrE, acrF, emrB, norE, tolC, marA, and soxS were determined by quantitative RT-PCR (LightCycler®) comparing induced vs. uninduced cultures (arabinose 0.1%).
Results: Compared with uninduced control, maximum induction of pCC1-lacrob resulted in an increase of: MIC to NFX: threefold (0.064 vs. 0.023 mg/L), MIC to CIP: no change; transcription of rob: 250-fold, mdfA 6.9-fold, acrAB 1.7-fold, emrB 0.6-fold, acrE 0.66-fold, acrF 0.69-fold, acrD 0.75-fold, norE 0.99-fold, marA 1.2-fold, soxS 0.66-fold, tolC 1.3-fold, and gapA 0.98-fold.
Conclusion: MdfA is a recently recognised efflux pump system in *E. coli* with preference for norfloxacin (1,2). We demonstrate that increased transcription of Rob from plasmid pCC1-lacrob resulted in increased transcription of mdfA but not acrAB and produces a correspondent phenotype with increased MIC to NFX but not CIP. Other efflux pump systems were only mildly affected (upregulation of acrAB, no effect for norE, downregulation of acrEF, emrB). The finding adds to the accumulating knowledge concerning the overlaps and parallels in regulation of efflux systems, and highlights their potential role in development of antimicrobial resistance.
References
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O281 Mechanisms of resistance to co-amoxiclav in clinical isolates of *Escherichia coli*

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Objectives: The aim of the study was to characterise the genetic basis of resistance to co-amoxiclav in clinical isolates of *Escherichia coli*.
Methods and Results: Six independent clinical isolates of *E. coli*, isolated from patients in hospitals in Cape Town (South Africa), were investigated. Of the six strains, four were isolated from urine, and one each was obtained from pus and blood. The MICs of co-amoxiclav (16–128 mg/L) for the strains indicated that they were resistant to this combination. All of the isolates were susceptible to cefoxitin and the newer oxyiminocephalosporins, suggesting that overproduction of AmpC was not responsible for the zco-amoxiclav resistance. To detect the presence of *blaTEM* genes in the isolates, PCR assays were carried out using primers to amplify the functional gene. A product of the expected size (1.2-kb) was obtained from each of the strains. Amplicons were either sequenced directly, or cloned into a vector prior to sequencing. Alignment of the nucleotide sequences with corresponding *blaTEM* sequences in the data bank enabled identification of the genes. *blaTEM-1B* was obtained from five strains. Of the five genes, four were associated with either of the strong promoters, Pa/b or P4, indicating increased expression of TEM-1. The strain containing the remaining *blaTEM-1B* gene, which was adjacent to the weak P3 promoter, harboured a second gene (*blaTEM-30A*), encoding an inhibitor resistant TEM (IRT). This gene was associated with the strong Pa/Pb promoter. An identical gene (*blaTEM-30A*) was identified in the remaining strain. To study the promoters used in the transcription of *blaTEM-30A*, primer extension studies were carried out. The fluorogram of the primer extension analysis indicated that mRNA transcription was initiated from three sites. The majority of transcripts emanated from the Pa component of Pa/Pb, indicating that of the three promoters upstream of *blaTEM-30A*, Pa is the most frequently recognised and therefore the most active promoter.
Conclusion: Resistance to co-amoxiclav in clinical isolates of *E. coli* was associated with either increased expression of *blaTEM-1B* or the presence of the IRT, *blaTEM-30A*.

O282 Loss of function mutations in *smeT* lead to multiresistance in *Stenotrophomonas maltophilia*

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Objectives: *Stenotrophomonas maltophilia* is an important, emerging, Gram-negative, nosocomial pathogen that can cause bacteraemia, respiratory tract colonisation and other organ-specific infections. Isolates are generally highly resistant to beta-lactams, macrolides and aminoglycosides, and have the potential to mutate to multidrug resistance (MDR), including to fluoroquinolones, tetracyclines and chloramphenicol. MDR has been linked to the overexpression of the tripartite efflux pump, SmeDEF in *S. maltophilia*, whose expression is believed to be controlled by the putative transcriptional repressor, *smeT*, encoded immediately upstream of *smeDEF*. The aim of this project was to confirm that SmeT is a repressor of SmeDEF expression, and that loss of function mutations in SmeT produce MDR.
Methods: *smeT* was amplified by PCR from the *S. maltophilia* isolate N531, and was manipulated using the *Hae*II restriction enzyme, T7 DNA polymerase and DNA ligase to result in a 4-bp deletion within the PCR amplicon. The *smeT2* mutant allele was ligated into the gene replacement vector pEXTc and the recombinant vector was transferred into *S. maltophilia* N531 by conjugation from *Escherichia coli* strain S17. A double recombination event, leading to replacement of wild-type *smeT* with the mutant *smeT2*, was selected for using sucrose, and transconjugants carrying the mutant *smeT2* allele were screened for by PCR followed by haeII digestion (which should be negative) of the product. MICs were determined by *E*-test, using Muller–Hinton agar.
Results: We were successful in introducing a 4-bp deletion within the *smeT* gene on the *S. maltophilia* chromosome, resulting in a frame-shift, and so loss of function mutation. MICs of a variety of antibiotics associated with the MDR phenotype were determined: i.e. tetracycline, chloramphenicol, erythromycin and ciprofloxacin. The wild-type strain had MICs of 1, 2, 6 and 0.38 mg/L, respectively for these drugs; the mutant had MICs of 6, 8, >256 and 32, respectively.
Conclusions: Loss of function mutations in *smeT* lead to greatly increased resistance to a variety of antimicrobials, but particularly of fluoroquinolones such as ciprofloxacin, in *S. maltophilia*. As such MDR mutants are frequently encountered, it is likely that smeT mutation is a common cause.

O283 Evaluation of differential gene expression in antimicrobial susceptible and resistant clinical isolates of *Klebsiella pneumoniae* by DNA microarray analysis

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Objectives: To seek for novel genes involved in antimicrobial resistance in *K. pneumoniae*, we used DNA chip technology to compare the gene expression profile of two clonally related strains isolated from the same patient (CSUB10S, porin+ efflux–; CSUB10R, porin– efflux+).
Methods: Total RNA from both strains was isolated, reverse transcribed, labelled with fluorescent nucleotides and hybridised to DNA microarrays displaying the complete genome of *Escherichia coli*. After hybridisation the microarrays were scanned and the intensity of the spots obtained for both samples were normalised and compared. Statistical analyses of five independent experiments identify those genes whose expression was significantly different between both strains. RT-PCR quantitation was used to verify the microarray data.
Results: Nineteen genes were upregulated in the resistant isolate compared with the susceptible isolate. No changes related to the AcrAB-TolC system were found. *ycjV* gene, which encodes for a transporter, was 4-fold up-regulated suggesting that this protein might contribute to antimicrobial resistance. By contrast, 33 genes were down-regulated in the resistant isolate, including 11 unknown genes and genes involved in the permeability of the cell envelope like ompC.
Conclusions: (1) *E. coli* DNA microarrays are suitable for *K. pneumoniae* gene expression profile analysis. (2) The number of genes down-regulated in the resistant isolate is higher that the genes up-regulated when compared with the susceptible strain. (3) *ycjV* is a candidate for a novel mechanism of antimicrobial resistance in *K. pneumoniae*.

O284 Molecular basis for trimethoprim resistance in clinical urinary isolates

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Objectives: To study the molecular basis for increasing resistance to trimethoprim in clinical, Gram-negative isolates. 105 urinary isolates partly selected for trimethoprim resistance were investigated regarding antibiotic resistance patterns, integron carriage, resistance gene cassettes and other resistance determinants.
Methods: Disc diffusion antimicrobial susceptibility test for 12 antibiotics and PCR for integrons of classes 1, 2 and 4 were performed on all isolates. Sequence analysis was used to investigate the cassette regions of 22 integrons and plasmid analysis was performed on selected isolates.
Results: Sixty-nine of the 105 isolates were resistant to trimethoprim. 53 isolates were resistant to at least four antibiotics, of these, 43 carried an integron. In total 65 integrons of classes 1 and 2 were detected in 59 isolates. Only one of the integron-positive isolates was susceptible to trimethoprim. As many as 10 isolates were resistant to trimethoprim and still negative for integrons. In the sequence analysis only *dfr* and *aadA* gene cassettes were detected despite the fact that the isolates were resistant to many more antibiotics than trimethoprim, streptomycin and spectinomycin. One new trimethoprim resistance gene, *dfr2d*, was also found. It appeared as a single gene cassette in a class 1 integron and it belongs to the unique group *dfr2*. The four genes in this group are completely unrelated to the remaining groups of about 15 *dfr*-genes, which are all identical to at least 20%.
Conclusions: Integrons constitute a vehicle for efficient spread of antibiotic resistance. These elements have the ability to acquire gene cassettes containing genes mediating resistance to many antimicrobials. Selective pressure from one antibiotic can co-select for genes mediating resistance to other antibiotics if they are assembled as gene cassettes in integrons. However, in this study, trimethoprim consumption alone seems to be high enough to select for integrons containing *dfr*-genes as the only advantageous gene for the bacteria. Nevertheless, integrons were shown to be more abundant in multiresistant isolates and these integrons are possibly carried by plasmids with many other resistance genes in addition to the integrated gene cassettes.

Paediatric infections and vaccines

O285 Analysis of rotavirus strains detected in 2003 in a Mantua hospital (Italy)

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Objectives: Rotaviruses are the major etiologic agents of infantile diarrhoea worldwide and the development of effective vaccines for rotavirus disease is considered a primary objective of the WHO. The main rotavirus serotypes G1, G2, G3 and G4 are the targets for vaccine development. However, unusual rotavirus serotypes, such as G5, G6, G8, G9 and G10 may acquire local relevance. The objective of the present study was to determine the antigenic specificities of the rotavirus strains spreading in Mantova (Italy), in 2003.
Methods: A total of 134 stool samples were tested for the presence of rotavirus by an immuno-chromatographic assay during 2003. Sixteen rotavirus-positive samples (11.9%) were detected from children between 6 months and 9 years of age (median age of 2.56 years). A 14-point score system was used to summarise the clinical severity. Rotavirus RNA was analysed by reverse transcription-PCR, PCR genotyping and sequence analysis for determination of the G (VP7) and P (VP4) specificity. In order to evaluate the correlation between serotypes and pathogenicity, a statistical analysis was performed by the General Linear Model procedure (SAS 1990).
Results: Eight samples were typed as G1, 4 as G4, 3 as G9 and one was not typeable. The VP7 genes of G9 strains revealed high genetic similarity to each other (about 100%) and to G9 strains identified recently in southern Italy (99.8%). Analysis of the VP4 gene revealed that all the strains (G1, G4 and G9) belonged to the P type P1A[8]. The severity of the disease was significantly higher for G9 rotavirus infections. The linear model fitted to pathogenicity score values was significant ($P < 0.0289$; $R^2 = 0.54$). Least square mean was 10.69 (standard error 1.20) for G9 rotaviruses and 6.32 (standard error 0.67) for the other serotypes.
Conclusions: The rotavirus strains were characterised as P[8], G1, P[8], G4 and P[8], G9. The VP7 gene of the G9 strains revealed a high genetic similarity to other Italian G9 strains and to the globally spreading G9 lineage described recently worldwide. Interestingly, it was possible to find a statistically supported correlation between the rotavirus serotype and the severity of the rotavirus-associated disease, as the symptoms induced by rotavirus G9 infections were significantly more severe. The results described here emphasises the role of rotavirus G9 as an epidemiologically important serotype and the need to include the G9 specificity in candidate rotavirus vaccines.

O286 Eye lesions associated with congenital toxoplasmosis: long-term follow-up of 327 children

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Background: Retinochoroiditis is the most frequent consequence of congenital toxoplasmosis. Early diagnosis and treatment are believed to reduce the risk of visual impairment. We report on the clinical evolution of ocular lesions and final visual function in a prospective cohort of congenitally infected children identified through monthly prenatal screening of pregnant women.
Methods: Of 1466 children born from 1453 mothers managed in our department for an acute maternal infection between 1988 and 2001, 327 born were eligible in our study: they had proven congenital toxoplasmosis and had been followed up for at least 6 months. Data on date of maternal infection, time and type of therapy, antenatal, neonatal and postnatal work ups, and ocular status were available for all of them and analysed.
Results: Most mothers had been infected during the second (27%) or third (67%) trimester. Pyrimethamine and sulphadiazine were given in utero to 38% of fetuses and after birth to 72% of newborns. Fansidar (R) was given for an average duration of 337 days ($\pm$ 23) in all but two children. After a median follow-up of 6 years (range: 6 months to 14 years), 71% of children were free of any lesions, 18% manifested no sequelae except retinochoroiditis and 11% had at least one none ocular sign (cranial calcification: 31 cases; hydrocephalus: six; microcephalus: one). Seventy-nine children (24%) had at least one retinochoroidal lesion, including a macular lesion in 31. Half of the initial lesions were diagnosed before 2 years of age and 76% before 5 years. In 23 of them (29%) at least one new event had been diagnosed up to ten years after detection of the first lesion. At the last examination, 55 children had lesions in one eye; of 45 children for whom data on final visual acuity were available, 31 (69%) had normal vision. Twenty-four children had lesions in both eyes; of the 21 for whom data on final visual acuity were available, 11 had normal vision in both eyes; none had bilateral visual impairment. 13 children had strabismus, four had microphtalmia, two had retinal detachment and one had cataract, hyalitis and optic nerve atrophy.
Conclusions: Clinicians, parents and elder children with congenital infection should be informed that late-onset retinal lesions and relapse can occur many years after birth but that the overall ocular prognosis of congenital toxoplasmosis is satisfactory when infection was identified early and treated accordingly.

O287 Quick resolving of unilateral middle ear effusion in acute otitis media monitored daily with tympanometry

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Objectives: Although duration of middle ear effusion (MEE) is one of the most important outcomes in the treatment of acute otitis media (AOM), little is known about the possibilities to measure it accurately and to minimise it. The aims of this study were to evaluate the duration of MEE in AOM treated by antimicrobials and to find factors influencing it.
Methods: Daily monitoring with tympanometry was performed by the parents among 90 children with AOM. They were 3 months to 7 years old (mean 3.1 years) and were randomly allocated to be treated with either oral amoxicillin (40 mg/kg/days) or cefuroxime-axetil (30 mg/kg/days) for 7 days. Daily monitoring was continued at least for 14 days or until the tympanometry was normal (curve A or C) in both ears. Clinical control with pneumatic otoscopy was carried out every 2 weeks.
Results: The disappearance of MEE took a mean time of 10.2 days from the diagnosis (median 7.5, range 1–58) among 75 successfully monitored patients. In two-thirds (69 %) of them MEE resolved in 14 days. The median duration of MEE did not differ significantly between the two treatment groups (8.0 vs. 7.0, $P = 0.7$). Ten children (13%) finished the monitoring with MEE still present, five of them at 14 days and another five at 29–43 days. The 23 (31 %) children with MEE lasting longer than 14 days had statistically significantly more often bilateral AOM at diagnosis and were younger than those recovering faster. The children who had unilateral AOM cured more rapidly than those with bilateral AOM ($P < 0.0001$ in log rank test, Figure 1). In logistic regression analysis adjusted for age, bilaterality explained the resolution of MEE at 2 weeks significantly (odds ratio 14.8, 95% confidence interval from 3.7 to 60, $P < 0.001$). Season, amount of previous AOM episodes, history of adenoidectomy or tympanostomy, sex, day care and parental smoking did not associate significantly with the cure rate.

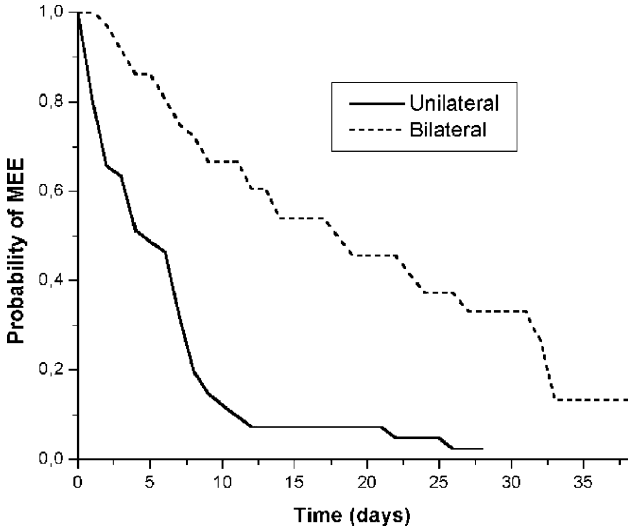

Figure 1. Disappearance of MEE after AOM in 75 children treated with antimicrobials by laterality at diagnosis.

Conclusion: Unilateral cases of AOM appeared to resolve much quicker than bilateral ones. This could be taken account when considering which of the AOM cases should be treated with antimicrobials and when planning control visits after AOM.

O288 M-serotypes, sensitivity to antibiotics and pyrogenic exotoxin genes of *Streptococcus pyogenes* invasive strains isolated in a Greek paediatric hospital during 1996–2002

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The reemergence of severe group A streptococcal infections (GAS), following many years of decreased incidence, was initiated during the late 1980s and is now described in all parts of the world. These infections, fatal in a significant rate, were caused by particular M-serotypes, especially M1. The aim of this study was to investigate the distribution of M-serotypes among GAS strains isolated from invasive infections of children.

Thirty-nine strains isolated from equal number of patients during a 7-year period (1996–2002) were tested. Eighteen of thirty-nine of the children were boys and 21 of 39 girls. Mean age of the little patients was 3.7 years (range: 1 day to 11 years). Samples from blood (32), skin lesions (3), pharynx (2), and pus (1) were cultured on blood agar 5%. The preliminary identification with bacitracin disk 0.05 U (OXOID) was followed by Lancefield serogrouping (TRANSLAB UKkit). The study was accomplished by serum opacity factor investigation, T-serotyping (anti-T sera from SEVAC Ltd, Prague), M-serotyping and spe genes (conventional and molecular techniques, RSIL, HPA, London). Sensitivity to antibiotics (penicillin, vancomycin, chloramphenicol, rifampicin, erythromycin, clindamycin), was also tested by disk diffusion method, double disk test for MLS resistance (Becton Dickinson's disks) and MIC (Etest, AB Biodisk). M1 (30.7%) was the predominant serotype, followed by M12 (17.9%), M4 (12.8%) and M89 (10.2%). Less frequent serotypes were M77 (7.7%) and M28 (5.1%). M6, M22, M73, M84, M90 and T5OF(+) shared the rate of 2.6% each. No strain was found resistant to penicillin, vancomycin, chloramphenicol and rifampicin. Seven of 39 (17.9%) isolates revealed resistance to macrolides (EryR). 5/7 EryR strains were of M phenotype (71.4%) whereas 2/7 (28.6%) of iMLS . Three of five (60%) of M phenotype strains were serotype M4 and the remaining two, M22 and M84. Strains of iMLS phenotype were M12 and M77. Eighteen of thirty-nine GAS strains were tested for pyrogenic exotoxin (*spe*) genes. *SpeA* was found in seven and *speC* in eight strains. As expected, all strains expressed *speB* gene. All M1 strains expressed *speA* gene. M1 was also the only serotype where *speA* gene was expressed. In conclusion, it seems possible that the most frequent GAS serotype causing invasive infections in children residing in Greece, is M1 (30.7%). Macrolide resistance rate with equal distribution over the years, was 17.9%, less than the one found in noninvasive strains (24%). As expected, M1 strains expressed *speA* gene.

O289 Penicillin for children affected with acute sore throat with or without group A beta-haemolytic streptococci in primary care

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Objectives: To assess the effectiveness of penicillin for 3 days and the treatment for 7 days in resolving symptoms in children with of sore throat as compared with placebo.
Methods: A randomised double-blind placebo-controlled trial was performed in 43 family practices in the Netherlands. A total of 156 children, aged 4–15 years, were included. They had sore throat for <7 days and matched with at least two of the four Centor criteria – that is, history of fever, absence of cough, swollen tender anterior cervical lymph nodes, and tonsillar exudate. Patients were randomly assigned to penicillin for 7 days, penicillin for 3 days followed by placebo for 4 days, and placebo for 7 days. The main outcome measures were duration of symptoms, mean consumption of analgesics, duration of not attending school, occurrence of streptococcal sequelae, eradication of the initial pathogen after 2 weeks, and recurrences of sore throat after 6 months.
Results: Penicillin treatment was not more beneficial than placebo in resolving symptoms of sore throat, neither in the total group nor in the 96 children with group A beta-haemolytic streptococci. In the 7-day penicillin, the 3-day penicillin and the placebo group 1, 2 and 8 children experienced a streptococcal sequela, respectively. These sequelae were an imminent peritonsillar abscess, impetigo and scarlet fever.
Conclusion: Our study showed no major beneficial effect of penicillin treatment in children with sore throat on average length of symptoms. Penicillin may, however, reduce streptococcal sequelae.

O290 Pertussis incidence in the Netherlands after introduction of an acellular booster vaccination at 4 years of age

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Objectives: In 1996–1997 different surveillance sources revealed an outbreak of pertussis in the Netherlands mostly among vaccinated children. In the following years the incidence of pertussis remained higher than in the period before 1996 and in 1999 another peak was observed. The high incidence of pertussis resulted in the introduction of an acellular booster vaccination for 4-year-olds in the National Vaccination Programme from October 2001 onwards.
Methods: To gain insight into the incidence of pertussis and into the effect of the introduction of an acellular booster vaccination for 4-year-olds in the Netherlands, surveillance data based on notifications, laboratory data, hospitalisations and deaths were analysed for 2001 and 2002, and compared with previous years.
Results: The reported incidence (per 100 000/year) in 2001 based on notifications (50.2), positive two-point serology (4.4), positive one-point serology (30.7) and hospital admissions (2.5), was comparable with 1999, but was higher than in 2002 (notifications

28.0, positive two-point serology 2.1, positive one-point serology 15.4 and hospitalisations 1.6). Although the total incidence in 2002 was slightly higher than in 2000 (26.6), the incidence in the age group vaccinated with the acellular booster vaccination had decreased with 45% compared with 2000. For the older age groups a slight increase in incidence was observed in 2002 compared with previous years. However, hospitalisations and severe disease still occurred mainly among young unvaccinated infants.
Conclusions: Pertussis is still endemic in the Netherlands with a higher incidence compared with the period prior to the epidemic in 1996–1997 and with a peak in the incidence every 2–3 years (1996, 1999, 2001). The introduction of an acellular booster-vaccination for 4-year-olds in 2001 has caused a decrease in the incidence of pertussis among the target-population itself. Long-term surveillance will be necessary to provide insight into the possible effect among the population at large. However, pertussis is still most severe among young unvaccinated infants. For the development of future vaccination strategies (e.g. boostering of adolescents and/or adults) more insight in the main sources of infection of young unvaccinated children in the Netherlands is therefore necessary.

O291 Intensified surveillance after introduction of vaccination against meningococcal C disease in the Netherlands

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Objectives: After introduction in 2002 of routine vaccination for children aged 14 months and a catch-up campaign for all 1–18 year olds (coverage ±94%)in June-November 2002 with the conjugated meningococcal C vaccine, an intensified surveillance of meningococcal disease was implemented in the Netherlands from January 2003 onwards. The aims of this intensified surveillance are to determine the remaining incidence and disease burden of meningococcal infections, to provide estimates of vaccine-efficacy, to identify vaccine failures and to gain insight into the role of early recognition in reducing disease severity.
Methods: A voluntary electronic questionnaire was linked to the mandatory notification system and notifications were combined with typing results from isolates of patients with meningococcal disease, collected by the Netherlands Reference Laboratory for bacterial meningitis.
Results: Incidence of meningococcal C disease decreased soon after the vaccination campaign had been carried out. In 2002 215 patients with meningococcal C disease were reported from January till November, while in the same period in 2003 only 37 patients had been reported. The largest decrease was observed among the vaccinated age groups. Among patients with meningococcal C disease those aged 1–19 years amounted yearly 58–69% in 2000–2002, while in 2003 this was only 19%. Since the introduction of meningococcal C conjugate vaccine, no cases of meningococcal C disease have been reported in children previously vaccinated. Remarkably, the number of patients aged 1–19 years with serogroup B also decreased (until November: 217 in 2000, 243 in 2001, 199 in 2002 and 137 in 2003). In January–November 2003 for 79% of notified cases the electronic questionnaire was completed. Further results will be available by the beginning of 2004. Conclusions: The vaccination campaign has had an immediate effect on the incidence of meningococcal serogroup C disease in the Netherlands, especially among vaccinated children. No vaccine failures have been reported so far. Surveillance of meningococcal disease remains necessary to evaluate long-term effects of the vaccination programme and will also give insight in the role of early recognition in reducing disease severity particularly for meningococcal group B disease for which no vaccine is available yet.

O292 Vaccination against hepatitis B in newborns of HBsAg positive mothers

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Objectives: Breakthrough infections after immunisation against hepatitis B, protective titres of anti-HBs antibodies after vaccination and vanishing of anti-HBs in long-term period were investigated in 576 neonates of HBsAg-positive mothers.
Methods: Combine passive–active immunisation was commenced in 1988. The number of immunised children gradually increased and the group included now 576 newborns. All mothers were HBsAg-positive during pregnancy, 30 of them (5%) were also HBeAg-positive. The children received hepatitis B immunoglobulin at birth and three 10 μg doses of plasma-derived or recombinant vaccine at interval 0, 1 and 6 months (only 21 children of HBeAg-positive mothers at interval of 0, 1 and 2 months). The immunisation schedules were completed in 534 children, serologic investigations after immunisation were performed in 489 children. Blood samples were obtained after completion of immunisation schedule, at 2 years of age, and biennially thereafter. Samples were tested by ELISA for HBsAg, anti-HBs and anti-HBc.
Results: Only two children became chronic HBsAg carrier, both suffered from mild, acute hepatitis B in the second year of life. One of them was infected with variants of HBsAg with substitutions at residues 137 and 139. Asymptomatic infections with presence of anti-HBc antibodies were observed in five children since fourth till eighth year of life. The increase of anti-HBs without revaccination was observed in 104 children. Anti-HBs antibodies were tested in 489 children after immunisation, protective titres of anti-HBs were proved in 471 of them (96%). Vanishing of protective anti-HBs antibodies were detected in 10, 29 and 37% children during second, fifth and tenth years of their life.
Conclusion: During 15-years monitoring in 576 children vaccinated against hepatitis B, were proved chronic HBsAg carrier status in two children, asymptomatic infections in five children, protective anti-HBs titres after immunisation in 96% children and vanishing of protective anti-HBs in 37% of them.
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O293 Protective effect in monkeys following immunisation with a live attenuated chimeric flavivirus against tick-borne encephalitis

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The attenuated chimeric virus Tp21/DEN4, containing genes encoding premembrane (*preM*) and envelope (*E*) glycoproteins of Langat virus in the backbone of Dengue four virus genome, was constructed by Dr Pletnev (Pletnev *et al*., 1998). It has been shown that chimeric virus Tp21/DEN4 has low neurovirulence and no neuroinvasiveness in immunodeficient mice (SCID). It protects immunocompetent mice against lethal challenge with a virulent strain of tick-borne encephalitis virus (TBEV) and is highly immunogenic and effective for protection against Langat virus in *Macaca mulatta* monkeys (Pletnev *et al*. 2001). Protective efficacy of the chimeric Tp21/DEN4 virus was evaluated in African green monkeys (*Cercophithecus aephiops*) against challenge with the highly virulent TBEV strain Abssetarov. Six of nine monkeys that received a single dose of 6.7–7.1 lg PFU of chimera developed a TBEV neutralizing antibody titre of 1:80 (GMT) at 2 weeks after inoculation, and all monkeys became seropositive after a second dose of inoculation that was administrated 35 days later. Two monkeys that received one immunization and three monkeys that received two immunizations with the chimera were challenged subcutaneously with 6.7 lg PFU of the highly virulent Absettarov strain of TBEV 5–6.5 weeks after the last immunisation and were

sacrificed on 35–37 days postchallenge. Control animals (two) were twice immunised with a commercial inactivated vaccine against TBEV. A 10-fold increase in NT antibody titre was observed in all monkeys on the second day postchallenge. Viraemia was very low, even in nonimmunized animals, and thus reduction of virus titres in blood could not be used to evaluate protective properties of the chimera in this species of monkey. TBEV challenge virus was not detected in submaxillary, axial lymph nodes and spleen of the monkeys immunised twice with the chimera or with inactivated vaccine; however, TBEV was detected in the organs of nonimmunized animals 3 weeks after inoculation. Therefore, a single immunization of Tp21/DEN4 chimera that contains structural *preM* and *E* proteins of Langat virus in African green monkeys elicits an immune response against TBEV. Double immunization with the chimera and the inactivated vaccine protects monkeys from the persistence of TBEV in peripheral organs.

O294 HLA polymorphisms and T cell cytokine responses to processed HLA class II measles virus peptides: the rationale for a measles peptide vaccine

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Objectives: Previously we have reported that HLA-DR molecules contribute the main restriction determinants for antigen-specific T-cell recognition of naturally processed measles virus (MV) class II epitopes derived from MV phosphoprotein (MV-P) and nucleoprotein (MV-N). We demonstrated a significant association of DRB1*0301 alleles with MV-P specific IFN-γ and IL-4 responses. In addition, DRB1*1501 and DRB1*1103/*1303 alleles were associated with MV-N induced IFN-γ and IL-4 secretion, respectively. Because of the tight linkage disequilibrium between DR and DQ and/or DP alleles and the frequent sharing of epitopes among these HLA class II loci, the importance of non-DR class II molecules in MV peptide responses should be understood.
Methods: We studied the association between cytokine responses and DQ and DP alleles among 313 (168 males and 145 females) children aged 12–18 years who received two doses of MMR-II vaccine. Genomic DNA was extracted from blood samples and used for HLA allele typing. PBMC were cultured alone or stimulated with peptides, and cytokines in supernatants were measured by ELISA. The allelic associations were determined by linear regression analysis.
Results: Overall mean IFN-γ and IL-4 secretion levels for MV-P and MV-N peptides were 99.3 compared with 13.2 pg/mL, and 0.3 compared with 4.1 pg/mL, respectively. Examination of IFN-γ responses to MV-P peptide indicated that none of the alleles of the DQ locus were associated with MV-P induced T-cell response. A marginally significant increase in the frequency of the DQB1*0201 ($P = 0.06$) and DQB1*0603 ($P = 0.10$) alleles was found among subjects who demonstrated high IL-4 levels to MV-P. DPB1*0201 ($P = 0.05$) and DPB1*1301 ($P = 0.10$) alleles provided suggestive evidence of an association with MV-P induced IL-4 secretion. DQB1*0602 ($P = 0.03$) allele provided the evidence of an association with MV-N induced high IFN-γ responses. By examining the DP alleles individually, we found a significant ($P = 0.006$) increase in the frequency of the DPB1* 0501 allele among subjects who produced low MV-N specific IL-4 responses.
Conclusion: We demonstrated that MV-derived peptides can be recognised in association with different HLA molecules, including DR, DQ and DP alleles. We suggest that non-DR class II molecules also restrict cytokine responses to naturally processed MV peptides. These results should be viewed cautiously due to multiple testing issues. This information is important for the design of new vaccines.

Tropical and parasitic diseases

O295 Post-travel related hospitalisation in Israel

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Objectives: Data regarding travel-related morbidity is still limited. In this study we evaluated the in-hospital diagnoses of travel-related diseases in the largest tertiary hospital in Israel.
Methods: Records were reviewed of all patients with a history of recent travel prior to their admission to The Sheba medical centre during 1 January 1999 to 30 November 2003. Demographic data, discharge diagnoses and travel destinations were recorded. In addition, demographics and destination of healthy travellers presenting to our clinic for pretravel consultation were recorded.
Results: During this period, 205 patients with travel-related infections were admitted. There were 144 (70%) males and 61 (30%) females; all were travellers except for 13 who were immigrants or foreign workers, most from Africa. Altogether 39 different diagnoses were recorded. A total of 159 patients (78%) were admitted with febrile diseases. The most common febrile diseases were: malaria in 54 (26%) patients, dengue fever in 27 (13%) and nonspecific (unidentified) febrile disease in 31 (16%) patients. Of the nonfebrile diseases, New World cutaneous lieshmaniasis (*L. braziliensis*) was the most common cause of admission (8%). Diarrhoeal diseases, which are the most common cause of morbidity during travel, accounted for only 11% of admissions. 99 (49%) had been to Asia, 69 (39%) to Africa, and 32 (16%) to the Americans. Although the distribution (statistics) of our healthy traveller population show that 70% travel to Asia, 25% to the Americas and only 5% to Africa. The major health problems of patients returning from Asia were dengue fever (27%), nonspecific febrile disease (18%), diarrhoeal diseases (13%), skin disorders (7%) and malaria (7%). The overwhelming majority of patients who had returned from Africa had malaria (59%), followed by nonspecific febrile disease (13%) and diarrhoeal diseases (10%). Most patients who were returning from Latin America suffered from lieshmaniasis (49%), *Plasmodium vivax* malaria (12%) nonspecific febrile disease (9%) and secondary-infected myiasis (9%).
Conclusion: Febrile diseases are the most common cause for post-travel hospitalization. Among them malaria, dengue fever and unidentified febrile disease were the most common. There were a destination-related diseases and travel to Africa is associated with higher rate of hospitalisations. Malaria and cutaneous lieshmaniasis had substantial male predominance, probably due to risk taking behaviour.

O296 Treatment of uncomplicated imported falciparum malaria in Italy

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Objectives: We compared a 3-day quinine-sulphadoxine/pyrimethamine regimen with mefloquine in travellers with acute falciparum malaria acquired in Africa.
Methods: Multicentre, randomised, oben-label trial. The efficacy end-points were the early cure rate, the rate of recrudescence during the 28 days of follow-up, the time required for parasite clearance, the time to clearance of fever and the time of hospital stay.

The tolerability end-point for the rate of emergence of adverse events during the follow-up period of the hospital stay.
Results: A total of 187 patients were enrolled from July 1999 to February 2003, of whom 93 were randomised to receive mefloquine (M) and 94 quinine-SP (Q). Immigrants and persons visiting relatives and friends represented 90% of all cases. The early cure rate was similar in the two groups: 98.9% (CI 97–100%) in M arm and 96.8% (93–100%) in Q arm. The extended follow-up was completed by 135 subjects (72.2%) with no recrudescences. There were no differences in the parasite clearance time, but patients in the M group had lower mean fever clearance time (35.9 h vs. 44.4 h, respectively, $P = 0.05$) and lower mean hospital stay time (3.9 days vs. 4.6 days, respectively, $P = 0.007$). The overall proportion of reported side-effects was similar in the two arms, but patients in the M arm had a significantly higher rate of central nervous system (SNC) disturbances (29.0% vs. 9.6%, $P < 0.001$).
Conclusion: Early and late cure rate were similar for a 3-day regimen of quinine-SP compared with mefloquine. Patients treated with mefloquine had a shorter fever clearance time and time of hospital stay.

O297 Retrospective study of imported malaria diagnosed in Mallorca, Spain

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Objectives: To evaluate the epidemiology, clinical and laboratory results of imported malaria diagnosed in Mallorca.
Methods: We included all malaria episodes diagnosed from January 1995 through November 2003 at the University Hospital Son Dureta by a positive blood smear. Species of plasmodia were identified by the blood smear and/or seminested multiplex PCR. Severe malaria was defined according to WHO criteria.
Results: A total of 79 malaria episodes were identified. We found *Plasmodium falciparum* in 54 episodes (68%), *P. vivax* in 10 (13%), *P. malariae* in two (3%), *P. ovale* in one (1%), mixed infection in four (5%), and unspecified plasmodia in eight (10%). Most (93%) *P. falciparum* infections were acquired in Africa, while five of 10 *P. vivax* infections where acquired in Latin America. Medical records were available for 65 episodes (82%); median age was 28 years (range 2–71 years), 66% were males. Eighteen patients (28%) were travellers and 47 patients (72%) were immigrants, of whom 22 reported recent travel (<1 year of malaria onset) to their country of origin. Thirty patients (46%), mostly immigrants, had a history of previous malaria. Among travellers, only two took chemoprophylaxis correctly, others took no prophylaxis ($n = 15$) or took it incorrectly ($n = 8$). Most patients (92%) were hospitalised. The median of duration of symptoms at diagnosis time was documented for 56 patients and was 3.5 days (range 0–30). The most common reasons for consultation included fever (86%), headache (51%) and chills (49%). Interestingly, nine patients (all immigrants) reported no symptoms at the time of diagnosis. At presentation, the following physical signs were present: fever (61%), lymphadenopathy (11%), hepatomegaly (42%), splenomegaly (44%), jaundice (11%), and haematuria (10%). Thrombocytopenia ($<150 \times 109/L$) was reported in 38 (58%) of patients followed by anaemia (haemoglobin levels below 12 g/dL) in 34 (52%) patients. Only two patients (3%) had severe malaria and no episode was fatal.
Conclusions: Most malaria episodes were caused by *P. falciparum* and involved immigrants who acquired malaria either before immigration to Spain or during a visit of their country of origin. We recommend performing a blood smear examination for malaria in this high-risk patient group. Enforcing adequate chemoprophylaxis during a visit of malaria-endemic areas, including immigrants, may represent an efficient strategy to prevent malaria.

O298 Ultrasound guided fine needle aspiration biopsy in the diagnosis of visceral Leishmaniasis

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Background: We report our experience with US-guided fine needle aspiration biopsy (FNAB) of the spleen in the diagnosis of visceral leishmaniasis in patients with negative sternum needle aspiration (SNAB).
Patients and methods: We reviewed clinical and imaging records of 16 patients (11 male; age range: 11–48 years) affected by visceral Leishmaniasis. All patients had previously undergone SNAB that had failed to show *Leishmania* parasites in all cases. The maximum spleen diameter was 14–21 cm (mean:17 cm). Blood scores were : platelets – 60.000 to 120.000 (mean 79.000); Prothrombin activity: 67–100% (mean 87%). We performed US guided spleen FNAB with a 22G × 9 cm length spinal needle.
Results: All the slides from spleen FNAB were positive for *Leishmania* amastygotes and all the cultures were positive for *Leishmania infantum* (sensitivity 100%).
Complications: The procedure was well tolerated in nine patients. Four patients complained mild to moderate pain in the upper left abdominal quadrant and showed haemoperitonaeum in the left subdiaphragmatic space at US (thickness 0.5–2 cm) within a few minutes from the spleen FNAB. Another patient, 24 h after the procedure, referred left thoraco-lumbar pain and showed left subdiaphragmatic blood collection (thickness 3 cm) without apparent lesions of the spleen capsule at US. All five patients only underwent US daily follow-up for 2–5 days that showed complete resolution of the peritoneal fluid collection. In no case blood transfusion was required.
Conclusions: US-guided spleen FNAB is an highly sensitive technique in the diagnosis of visceral leishmaniasis (100% in our experience). However, it can cause haemoperitonaeum also in patients with slight alteration of coagulation parameters (five of 16, 31% in our series), therefore, it should always be performed only in case of negative result of SNAB.

O299 Short-course liposomal amphotericin B in the treatment of infantile visceral leishmaniasis: the Italian experience

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Objectives: To evaluate in a retrospective analysis the efficacy and the safety of a short course of liposomal amphotericin B (L-AmB) in infantile cases of Mediterranean visceral leishmaniasis (VL) diagnosed over a 10-year period in Italy.
Patients and methods: Patients diagnosed as having VL consecutively admitted from December 1992 to December 2001 at four main referral childrens' hospitals of Italy and treated with six intravenous doses of 3 mg L-AmB/kg given on days 1–5 and 10 (a total dose of 18 mg/kg) were included. Demographic data, nutritional status, underlying diseases, clinical and laboratory findings and therapy outcome were considered.
Results: A total of 164 HIV-negative children (median age 1.6 years, range 4 months–14 years) were enrolled. All the patients responded clinically to treatment and did not present adverse events related to drug infusion. Seven patients (4.3%) had a clinical and parasitological relapse 3–15 months after therapy. All relapses were successfully retreated with 3 mg L-AmB/kg for 10 days consecutively (a total dose of 30 mg/kg).
Conclusion: This study highlights the efficacy (>95%) and safety of the 6-dose L-AmB regimen and validates it as a first-line treatment for Mediterranean visceral leishmaniasis in children.

O300 An open, randomised controlled trial of penicillin, doxycycline, and cefotaxime in patients with severe leptospirosis

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Objective: To compare the efficacy and safety of parenteral doxycycline, cefotaxime and penicillin in the treatment of severe leptospirosis.
Methods: Between July 2001 and December 2000, an open label randomised study in patients with suspected severe leptospirosis was conducted in 540 patients admitted to four hospitals in North Eastern Thailand; Udornthani Hospital, Udornthani Province; Maharaj Nakhon Ratchasima Hospital, Nakhon Ratchasima Province, Loei Hospital, Loei Province and Ban Mai Chaiyapod Hospital, Bureerum Province.
Results: A total of 252 (47%) patients had serological or culture confirmed leptospirosis. Overall mortality was 4.8%. There were no significant different between the antibiotics in mortality, defervescence, or time to resolve laboratory overall, or in the subgroup of patients with confirmed leptospirosis. Rickettsial infection was diagnosed in 132 patients, and in these patients doxycycline was superior to penicillin.
Conclusion: Doxycycline or cefotaxime are satisfactory alternatives to penicillin for treatment of severe leptospirosis.

O301 A study on some epidemiologic and paediatric aspects of toxocarosis in Hungary

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Objectives: Toxocarosis is the most frequent human helminthosis in Hungary. Most of the clinical symptoms are nonspecific, thus, its diagnosis depends considerably on laboratory methods, such as ELISA and Western blot (WB) techniques. The objectives of our study was to determine the frequency and distribution of *Toxocara* seroprevalence in the different regions and the different age groups of Hungary. Special reference will be given to children in whom anti-*Toxocara* antibodies were determined for differential diagnostic reasons, such as exanthemata and/or asthmatic symptoms.
Methods: Sera were obtained from 6985 asymptomatic individuals representing all age groups of the population of Hungary distributed to 20 different regions. Sera of 1427 symptomatic children aged <1–14 years were also obtained from different regions of Hungary. Seroprevalence of the symptomatic children were compared with 1605 asymptomatic children. *Toxocara* seroprevalence was measured with NOVATEC *Toxocara* ELISA IgG kit. In case of negative or low-positive values obtained by ELISA, the results were confirmed by WB method (LDBIO).
Results: The sera of 1977 persons of 6985 asymptomatic individuals were found to be positive for *Toxocara* IgG antibody (28.3%). The lowest prevalence (17.6%) was found in Budapest, the highest ones were found in Szabolcs-Szatmár-Bereg (39.4%) and Hajdú-Bihar (38.2%) countries. Seropositivity rapidly increases by age reaching 37.0% at the age of 10–14 years. A significantly higher percentage (31%, $P = 0.01$) of *Toxocara* seropositivity was found in children suffering from bronchial asthma when compared with the 17% seropositivity measured in asymptomatic control groups of children. The borderline anti-*Toxocara* IgG ELISA results were found to be positive with WB method.
Conclusions: In Hungary, 28.3% of the population has anti-*Toxocara* IgG antibodies. The highest positivity (39.4%, 38.2%) were found in the most under-developed regions of Hungary, the lowest one (17.6%) was found in the capital. Probably, this reflects the differences in the hygienic conditions. The *Toxocara* seroprevalence rapidly increases by the age. This can be explained by the frequent contact of children with contaminated soil. A significantly higher percentage of *Toxocara* seropositivity was found in asthmatic children compared with the asymptomatic children. The Western blot technique is very useful in confirming the borderline and negative anti- *Toxocara* IgG values obtained by ELISA method.

O302 Serological evidence for the occurrence of tick-borne diseases in northern Mongolia

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Objective: The aim of our study was assess the seroprevalence of antibodies against the TBE virus in patients from northern and central Mongolia.
Methods: During August 2003, 223 serum samples were drawn from volunteers of Selenga aimag, 110 serum samples were drawn from volunteers of Bulgan aimag and central aimag each. They were screened by a commercially available IgG ELISA (Dade Behring) and positive samples were subjected to a neutralisation assay. Samples, which yielded a neutralisation at 1:10 or higher were rated as positive.
Results: Eleven patients from Selenga (4.7%) and one patient from Bulgan (0.9%) were found seropositive against TBE virus. No sample from central aimag was found positive. Neutralisation titres lay between 1:10 and 1:60. About 20% of the patients reported a tick bite, which were usually acquired in the northern part of the country.
Conclusions: Tick-borne encephalitis is likely to exist in the northern parts of Mongolia, where *Ixodes scapularis* occurs. In the central parts of the country, serological findings do not support the occurrence of this disease.

O303 The United Kingdom National External Quality Assessment Scheme for Parasitology: *Toxoplasma* Subschemes

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Objectives: The *Toxoplasma* Serology Subschemes are operated from the department of Clinical Parasitology, Hospital for Tropical Diseases, London in collaboration with the *Toxoplasma* Reference Laboratories in Inverness and Swansea and the Department of Medical Microbiology, St George's Hospital, London. The subschemes consist of toxoplasma serology and toxoplasma IgM are designed to provide information allowing participants to gain an insight into their performance in the detection of *Toxoplasma* antibodies and to take individual action to investigate and remedy any discrepancies. Participation is open to UK and overseas microbiology laboratories.
Methods: Serum samples for the detection of *Toxoplasma* IgG antibodies are distributed to 312 laboratories and to 213 laboratories for the detection of *Toxoplasma* IgM antibodies. Following analyses of results, a full report is provided for all participants. This includes specific comments on the *Toxoplasma*-specific antibody content of each specimen and, for one specimen, its relevance to the clinical problem. Serum samples, pre and postdistribution testing of samples and teaching information are provided by the *Toxoplasma* Reference Laboratories in Inverness and Swansea and the Department of Medical Microbiology, St Georges Hospital, London.
Results: In general, participants experience very few problems with an average of 98% of participants obtaining the correct result for *Toxoplasma* IgG detection since 1993 and 95% obtaining the correct results for *Toxoplasma* IgM detection since 1999.
Conclusion: Although participants experience very few problems, the *Toxoplasma* subschemes have highlighted some problems with the detection of *Toxoplasma* antibodies. The most common problem

is failure to detect low positive titres (i.e. dye test 16 IU/mL and Eiken latex agglutination test of 1:16) by many participants, which were related to participants deviating from the manufacturer's instructions as noted from the range of titres and cut-off titres reported. These specimens highlighted the importance of adhering to the manufacturer's instruction and choosing the correct assay for the clinical group of interest.

O304 Web-based guidelines for the evaluation of fever in returning travellers and migrants (http://www.fevertravel.ch): promotion and appropriateness for the primary care physician

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Background: Fever upon return can be caused by diseases that are rapidly fatal if left untreated. The differential diagnosis is wide. Physicians often lack the necessary knowledge to appropriately take care of such patients.
Objectives: To develop, promote and assess practice guidelines for the first evaluation of patients presenting with fever upon return from a tropical or subtropical country.
Methodology: After a systematic review of the literature, a decision chart was constructed and extensively discussed with a national and international panel of experts in travel/tropical medicine, specialists in infectious diseases and internal medicine as well as by private practitioners. After publication (*J Travel Med* 2003; **10** Suppl. 2), a website was created and is now freely available on the internet for the medical personal who want to use the guidelines (http://www.fevertravel.ch). Presentations and tutorials were run at national and international travel/tropical and internal medicine meetings to promote the use of the guidelines. At the same time, a pilot study was conducted at the Medical Outpatient Clinic to assess the feasibility and safety of the guidelines when used in the context they have been designed for. Physicians on call were asked to use the decision chart when caring for travelers or migrants with fever upon return. Navigation through the decision chart was recorded. Diagnostic tests performed, treatment administered, initial and final diagnosis and final outcome were collected prospectively. When the proposed attitude was not followed, reasons for nonadherence were investigated.
Results: From April to November 2003, 1775 visits were made to the website, mainly in Europe and the USA. Since study initiation, 53 physician/patient pairs have been included. Results on this first sample show that 51% were fully adherent to our guidelines. The main reasons for nonadherence were no repetition of malaria tests (nine of 23), no chest X-ray in case of cough (seven of 13) and no presumptive treatment for fever + diarrhoea (four of 11), all in the absence of alternative documented diagnosis.
Conclusion: Although considered very useful by our primary care physicians, the guidelines will need refinements following investigation on a larger sample size. Infectious disease specialists who work in collaboration with primary care or emergency physicians are welcome to join the study when internet support for complete recording of the path followed by the user will be available.

Novel advances in antifugal therapy (Symposium arranged Vicuron)

S309 The relevance of pharmacokinetics in the treatment of fungal infections

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Serious fungal infections affect patients with compromised immune systems and are often fatal despite current treatments. A major concern when treating serious fungal infections is the agent's ability to eradicate the fungi responsible for infection rather than simply inhibit or slow its growth. Most importantly, empiric therapy requires broad-spectrum agents that can eradicate a wide range of fungi that cause infection. An entirely new class of antifungals, the echinocandins, have a novel mode of action. Three new compounds in this class (caspofungin, anidulafungin, and micafungin) appear to be potent *in vitro* against the fungi most often responsible for serious systemic infections, *Candida* and *Aspergillus*. These compounds are noncompetitive inhibitors of (1,3)-a-D-glucan synthase, an enzyme involved in the synthesis of glucan, which is the major component of the cell wall of many fungi. The echinocandins are different with respect to their pharmacokinetic and pharmacodynamic properties, however trials comparing these agents are not currently available. Anidulafungin is chemically degraded, not metabolised through the cytochrome P450 system, and not excreted renally. Other members of the class, caspofungin and micafungin, are also chemically degraded, but in contrast to anidulafungin, undergo some hepatic metabolism. Chemical degradation may allow for fewer of the drug interactions and side effects seen with the azole class. In general, the echinocandins have improved antifungal activity and in particular, anidulafungin is highly effective both in terms of breadth of spectrum and potency as compared with natural echinocandins. Anidulafungin is a broad-spectrum agent that has shown potency *in vitro* against *Candida* and *Aspergillus*. Preclinical studies have shown that a 5 min exposure to anidulafungin *in vitro* kills more than 99% of *Candida*, including fluconazole-resistant strains. Early clinical studies have suggested an improved safety profile compared with that of available agents and the lack of drug-to-drug interactions (1). In addition, no dose modifications are required for anidulafungin in patients with any degree of hepatic and or renal impairment (2). It is important to note that the application of pharmacodynamic principles to antifungal drug therapy of *Candida* infections has provided an understanding of the relationship between drug dosing and treatment outcome, which is similar to that observed for antibacterial pharmacodynamics. Initial observations of the pharmacodynamics of triazoles have correlated with the results of clinical trials and have proved useful for validations of *in vitro* susceptibility breakpoints (3).

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S310 Emerging epidemiology of fungal infections

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Over the past decade, changes have occurred in the patterns of *Candida* species and infections and in fungal pathogen dominance. From 1980 to 1990, *Candida albicans* was very common, accounting for 61% of nosocomial fungal infections. *C. glabrata* accounted for approximately 8% of such infections that were reported to the Centers for Disease Control and Prevention in the USA (1). Other organisms, like *Aspergillus*, also cause invasive mycoses and are difficult infections to manage especially since they target immunocompromised patients. Data collected from surveillance programs in the USA during the 1990s demonstrate that *C. albicans* is still the primary pathogen. *Candida glabrata* is now the second leading cause of candidal bloodstream infections in the USA (2). The use of low-dose fluconazole may be contributing to the emergence of nonalbicans *Candida* species (NAC), most notably *C. glabrata* and

C. krusei. A current limitation to fluconazole use is its *in vitro* activity against NAC species. NAC species are emerging as a growing cause of invasive candidiasis in all immunocompromised patient groups. This knowledge makes the initial antifungal drug choice critically important. The low minimum inhibitory concentrations (MICs) of echinocandins against a broad spectrum of *Candida* species offer great potential in treating invasive *Candida* infections. Recently published guidelines from the Infectious Disease Society of America (IDSA) review strategies for treatment of most forms of invasive candidiasis (3). In general, amphotericin B-based preparations, the azole antifungal agents, and the echinocandin antifungal agents play a role in treatment, whereby the proportions of patients to be treated for systemic infections with polyenes is decreasing continuously.

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S311 Anidulafungin, a new echinocandin and its role in the management of invasive *Candida* infections

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Anidulafungin is a novel echinocandin that has demonstrated potency *in vitro* and *in vivo* against medically significant fungi, including *Candida* and *Aspergillus* (1). Consistent with its mechanism of action, anidulafungin is fungicidal for *Candida* spp. and there is no cross-resistance with older classes of antifungal agents (e.g. azoles, polyenes) that have different molecular targets. Isolates of *Candida* that are resistant to azoles or amphotericin B exhibit no cross-resistance to anidulafungin. To date, resistance to anidulafungin has not been detected *in vitro*, in animal infection models, or in the clinic. Pharmacokinetic (PK) studies indicate that anidulafungin has predictable PK. The half-life in humans and most animal species is 1 day, which allows for once-daily dosing in the clinic. PK data also show that anidulafungin dosage adjustments are not required for adult patients on the basis of age, weight, gender, race/ethnicity, hepatic or renal impairment (including haemodialysis), or concomitant medications. Clinical studies of anidulafungin are evaluating its safety and efficacy in the treatment of patients with oesophageal candidiasis, candidemia, and other forms of invasive candidiasis, azole-refractory mucosal candidiasis, as well as invasive apergillosis. This antifungal has proven to be highly effective in eradicating *Candida* spp. in clinical studies of oesophageal candidiasis and invasive candidiasis/candidemia. Anidulafungin was well tolerated by patients in a large comparative phase 3 oesophageal candidiasis study and a phase 2 invasive candidiasis study. Results of a phase 1 drug–drug interaction study demonstrate that the combination of anidulafungin and cyclosporin A may be administered safely and may not require dosage adjustment of either drug (2).

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S312 Contemporary therapy approaches for invasive aspergillosis

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Medical advances such as newer, aggressive chemotherapy, the frequent use of bone marrow transplantation, and organ transplantation have given rise to an increased incidence of invasive aspergillosis (IA) that parallels the increase in immunocompromised patients. The incidence of invasive mould infections following hematopoietic stem cell transplantation has tripled (1) with *Aspergillus* species the most common pathogen. For decades, the treatment for IA has been relatively toxic. Amphotericin B is only moderately effective against IA and causes significant nephrotoxicity and infusion-related toxicity (2). Voriconazole has recently been shown to be superior to amphotericin B in primary therapy of IA (3). However, nearly half of the patients are unresponsive to voriconazole and further improvement in therapy is needed. Currently, there are many new antifungals under development or in clinical trials, including new classes of drugs with novel targets such as the echinocandins. The activity of echinocandins against *Aspergillus* species has been studied for over a decade and its potential use in aspergillosis has been documented. In particular, the activity of anidulafungin against *Aspergillus* spp. has been demonstrated under a variety of growth conditions. As is the case for *Candida*, the apparent MICs of anidulafungin for *Aspergillus* vary with the culture medium. In recent studies, potentially useful synergy between anidulafungin and itraconazole or voriconazole was observed against *Aspergillus* isolates (4) and lack of antagonism was seen with amphotericin B (5). The use of newer antifungals and the potential synergistic effect of antifungal combinations, possibly with cytokine therapy, provides a basis for improved treatment strategies to combat IA.

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Meningococcal infection: still a challenge in the 21st century

K323 Meningococcal infection: still a challenge in the 21st century

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Invasive meningococcal disease (IMD) is still one of the most dangerous infectious diseases, despite early antibiotic treatment and development of modern intensive care for the patients. The National Reference Laboratory (NRL) for Meningococcal Infections in Prague has been dealing with this disease since the 1970s using a multi-discipline approach to the study of the causative agent and host factors. The NRL has a collection of 3500 *N. meningitidis* strains isolated from both patients with IMD and healthy carriers over a period of more than 30 years. Clonal analysis of meningococcal populations by multilocus sequence typing (MLST) identified hypervirulent complexes which are spread worldwide and cause severe IMD with a high case fatality rate. The international collaborative studies showed differences between meningococcal populations causing IMD and the carriage lineages. The meningococcal population from carriers was highly diverse and most nucleotide sequence diversity resulted from the reassortment of alleles by horizontal genetic exchange (*J Clin Microb* 2000;38:4492). Hypervirulent complex ST-11 of *Neisseria meningitidis* C emerged in the Czech Republic in 1993 and caused an increase in morbidity and case fatality rate of IMD. We developed a method for MLST directly from clinical specimens (*Epidemiol Infect* 2002;128:157) which allows more precise surveillance of

IMD. Recently, hypervirulent complexes of serogroup B (ST-32 and ST-18) have started to replace ST-11 and to cause a high case fatality rate of IMD as well. Higher and quicker adhesion to epithelial cells with lower phagocytosis as revealed in strains isolated from IMD compared with those from healthy carriers (*Microbiologica*, 2000;23:185) also confirmed the difference between these meningococcal populations. Analysis of correlation between socioeconomic and indoor enviroment factors and risk of IMD showed strong association of IMD with smoking (*Arch Dis Child* 2000;83:117). Hypervirulent complex ST-11 which emerged in the Czech Republic in 1993 caused an increase in antimeningococcal herd immunity among healthy population against this meningococcal complex (*Epidemiol Infect* 1999;123:193). However, naturally occurring antimeningococcal antibodies are not able to stop the spread of the hypervirulent complex in the population. A strategy of vaccination targeted to the part of population at highest risk of IMD was adopted in the country.

Cancer and infection: a two-way road (Joint symposium arranged with IATG-EORTC)

S328 Tuberculosis and stem cell transplantation

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Tuberculosis (TB), once accepted as a disease that seemed uncurable, lost its importance in the last decades of the 20th century. Tuberculosis is reemeerging in recent years. The main reasons for this problem are weakened TB control programs, HIV/AIDS and immigration and drug resistance. According to the reports of the WHO; one-third of the world's population has latent TB, 9 million cases of active TB emerge annually, causing 2–3 million deaths. Most new cases occur in the most populated nations such as India and China. Although the incidence of tuberculosis declined in North America and Western Europe, case rates have increased over the past 10 years. High rates of TB is inevitable in Stem cell transplantation (SCT) patients because of impaired cellular immunity, T-cell depletion, GVHD, Corticosteroids, high dose chemotherapy/TBI and the underlying disease. When compared with the solid organ transplants, TB rates are not as high as expected in SCT (0.16–2.29%) maybe due to the facts that duration of immunosuppression is not very long and most SCT are performed in developed countries where Mycobacterium infections is low. In the autologous transplants, TB has the same rate with the population. In allogeneic setting most of the cases are diagnosed +100 days posttransplant and acute GVHD is common (64%). Extrapulmonary TB is not infrequent (15%) and classical pulmonary manifestations of TB are rare. Nonspesific pulmonary findings are frequent. Mortality is high (18.5%) and most of the cases can be diagnosed and treated before death (90.7%). Prophylaxis may bring a reduction in TB cases but there will be toxicity related to the prophylactic agents. A study by Arslan *et al*. showed that INH prophylaxis in SCT patients reduced the rates of TB with an acceptable toxicity. Prophylaxis can be employed in endemic regions and has to be reserved only for PPD (+) patients or patients with a previous history of TB. As a conclusion; TB rates in SCT are parallel to the TB rates in a given population, endemic TB countries have highest TB rates, there are some ill defined risk factors such as previous TB history, positive PPD, GVHD, T-cell depletion and, corticosteroids. There is no need for prophylaxis in autologous SCT. There is a satisfactory response to standart anti-TB therapy, but mortality is still high, early diagnosis improves response to anti-TB therapy and drug resistant TB is not a problem at this moment.

Vector-transmitted diseases in the tropics

S330 Epidemiology of imported malaria in Europe

T. Jelinek for TropNetEurop

Malaria continues to cause high morbidity among European travellers. A thorough recording of epidemiological and clinical aspects of imported malaria has been shown to be helpful for detecting new outbreaks and areas of developing drug resistance. Data from national surveillance systems in Europe vary greatly in terms of quality and reliability. It has been estimated that coverage rates are at or below 50% of actual cases. In particular imported malaria through immigrants, many of them being illegal, are rarely reported. Since there is a general lack of surveillance data for imported infectious diseases in Europe, the European Network on Imported Infectious Disease Surveillance (TropNetEurop), an electronic network of clinical sites related to imported infectious diseases was founded in February 1999. The network is designed to effectively detect emerging infections of potential regional, national or global impact at their point of entry into the domestic population. From the beginning, malaria has been one of the major targets within this network of 46 clinical sites throughout 16 European countries. Judging from the data provided by national systems of disease notification, TropNetEurop covers approximately 12% of all malaria patients seen in Europe. Reports from immigrants and European patients with falciparum malaria are continuously analysed for epidemiological information and clinical features. Data from individual European regions are quite diverse, reflecting the local pattern of immigrants and the amount of international travel in the local population. By far the most infections are being imported from West Africa, suggesting a high risk for falciparum malaria for travel to that region. Data show that Europeans or other nonimmune travellers suffer more complications during the clinical course of the disease. Consequently, deaths occur almost exclusively in this group. Data reported by member sites of TropNetEurop can contribute to the understanding of epidemiology and clinical characteristics of imported falciparum malaria.

S331 Filarial infections – a risk for travellers?

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Basle, CH

Filarial infections present with lymphatic (Wuchereria, Brugia), cutaneous (Onchocerca, Loa, Dirofilaria, Drancunculus), ocular

(Loa, Dirofilaria) or pulmonary (Dirofilaria) manifestations. Symptomatic infections usually occur after repeated insect bites, or exceedingly rarely by ingestion of infested water (Dracunculus), in endemic areas. Thus, these infections are rarely seen in travellers. Long-term travellers and expatriates living in infested areas may be affected, but the most likely persons to be diagnosed in industrialised countries with such an infection are migrants who have been living in their endemic countries of origin. The low risk for travellers is reflected in the scientific literature, revealing mainly anecdotal reports among people returning from endemic areas. However, the possibility of treating some of these rare, often long-standing infections with effective drugs such as ivermectin, diethylcarbamazine and albendazole render the diagnosis important. Finding the parasite or antibodies against the various worms should be in the hands of experts who can also advise general practitioners on their geographical distribution and on their clinical features. The disease management approach for the returned traveller or migrant with filarial infections is often different from the approach taken for filarial-infected residents in endemic countries.

S332 Imported and autochthonous Leishmaniasis

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Madrid, E

The *Leishmania* genus is constituted by two dozens of human pathogenic species, causing three major clinical forms: cutaneous, mucocutaneous and visceral leishmaniasis, with specific geographical distribution along 88 endemic countries. All *Leishmania* species share a typical metaxenic cycle but each with given vertebrate reservoirs, and sand fly vectors, conditioning endemic or epidemic transmission. A re-emergency of leishmaniasis in different areas of the world is proven due to agriculture and irrigation programmes, urbanisation, deforestation, population displacements, famine, introduction of infected reservoirs or sand fly neo-colonisations, human behaviour changes and international mobility, aspects that are anlysed in this paper. Emphasis is paid to Mediterranean leishmaniasis updating some epidemiological features on HIV–*Leishmania* co-infection and its transmission when sharing contaminated syringes, blood banks, the capability of infected dogs (asymptomatic or symptomatic) to transmit the parasite to sand flies both before and after chemotherapy, and leishmaniasis imported by (or exported to) international travellers.

The clinical relevance of rapid diagnostics (symposium arranged with ESGMD)

S334 Real-time PCR detection of group B streptococci (GBS) in less than 1 h: a clinical revolution

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Group B streptococci (GBS) are an important cause of neonatal sepsis and meningitis. Implementation of selective intrapartum chemoprophylaxis based on either a screening-based approach or a risk-based approach has led to a substantial decrease in the morbidity and mortality of GBS disease. Current 'gold-standard' detection methods for GBS are selective broth cultures of combined vaginal and anal specimens collected at 35–37 weeks gestation. Rapid immunological detection assays, including latex agglutination-based test, are available. These methods are useful in rapid identification of heavily colonised women, but are unable to detect light GBS colonisation due to poor sensitivity. Recent development of real-time PCR and fluorescence labelling technologies has provided new detection platforms for bacterial identification. Combining appropriate, simple and very rapid sample preparation and DNA extraction methods with real time PCR, our research group in collaboration with Infectio Diagnostic (IDI) Inc. have developed, fabricated and commercialised the first FDA approved real time PCR assay that can identify in 30–45 min GBS in pregnant women in labour. Stat microbiology is here! The application of these assays in the current prevention strategy recommended by CDC, will simplify the prevention practice and rationalise antibiotic use. This rapid GBS DNA based theranostic test as well as others in development will revolutionise clinical practice by providing the physician with appropriate microbiology information that will immediately orient the management of his patient.

S335 Rapid detection of respiratory viruses: when and how?

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Edegem, B

The development and transnational spread of bacterial antibiotic resistance is an increasing source of concern and can be reduced if proper and rapid diagnostic tests can be made available for the aetiologic diagnosis of infections. Particularly in the diagnosis of lower respiratory tract infections (LRTI), the lack of a clear distinction between bacterial and viral aetiologies is a permanent cause of difficult diagnostic and therapeutic decisions. The development of more rapid diagnostic methods might allow better targeting of antimicrobial treatments with minimisation of unnecessary use of these drugs. For the detection of respiratory viruses conventional culture techniques are still considered as the gold standard. However results are mostly available too late to have an impact on patient management. Newer nonamplification methods have become available, e.g. enzyme immunoassays, optical immunoassays, agglutination assays and immunofluorescence. Advantages and limitations of these assays will be discussed. The newest developments include appropriate DNA and RNA based amplification techniques (both NASBA and PCR) for the detection of an extended number of agents responsible for LRTI. To increase the diagnostic value of the tests, multiplex formats were developed. Their sensitivity and specificity compared favourably with the corresponding mono formats. Real time amplification, the latest technical progress, produces, within a considerable shorter time, results with a lower risk of false positives due to cross-contaminations. As results can be obtained within the same day, patient management with appropriate therapy or reduction of unnecessary antibiotic therapy in LRTI will be possible. A number of technical aspects of these amplification assays, and their advantages will be discussed. Indications for the use of these rapid techniques in different clinical situations will be discussed. For the clinical implementation of these tests, a compromise must be found between test sensitivity, turnaround time and cost.

S336 Cost-effectiveness of real-time PCR for respiratory viruses in patients hospitalised with lower respiratory tract infection

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Objectives: Because of their sensitivity nucleic acid amplification techniques (NATs) are potentially very attractive for early, rapid diagnosis of lower respiratory tract infections (LRTI). However, costs of NATs are high and may preclude their implementation in routine diagnostic practice. We studied the cost-effectiveness of NATs for rapid diagnosis of LRTI.

Methods: Between November 2002 and May 2004, nose–throat samples were taken from immunocompetent patients hospitalised for antibiotic treatment of LRTI. The samples were evaluated by virus culture and by real-time PCR's for Adenoviruses, Coronaviruses, Influenzavirus A/B, Parainfluenzavirus 1-4, Rhinoviruses, RS virus and *Chlamydia pneumoniae*, *Legionella pneumophila* and *Mycoplasma pneumoniae*. Patients were randomised into one of two arms, in only one of which PCR results were made available within 24–48 h to allow modification of patient management. In addition, blood and sputum samples were taken for bacterial cultures and serology for respiratory viruses and atypical pathogens.
Results: Sxity-three consecutive patients (34 men, age 63 ± 16) were included; 29 (34%) had pneumonia (chest X-ray showing infiltrate). Respiratory viruses were detected by culture in eight patients, by real-time PCR in 19 patients (30%). Most frequently detected agents were Influenzavirus A ($n = 9$) and Coronavirus ($n = 5$). In one patient Influenzavirus A was cultured, but not detected by real-time PCR. In 14 patients (22%), the virus detected by real-time PCR was the only aetiologic agent identified. In these patients, antibacterial treatment could have been withheld. An average standard 10-day course of antibiotic treatment for a for LRTI costs approximately 450 €. Thus, real-time PCR can reduce antibiotic costs by $14 \times 450 = 6300$ € in 63 patients. In practice, however, physicians were reluctant to discontinue antibiotic treatment after a positive PCR. Conclusion Antibiotic use may be reduced using real-time PCR to rapidly identify viral LRTI. To achieve such reduction, physicians should be willing to discontinue antibiotic treatment in case of positive PCR-results and negative routine cultures.

The international dimension of epidemiological typing (Symposium arranged with ESGEM)

S337 The easy visualisation of clones from multilocus sequence typing data

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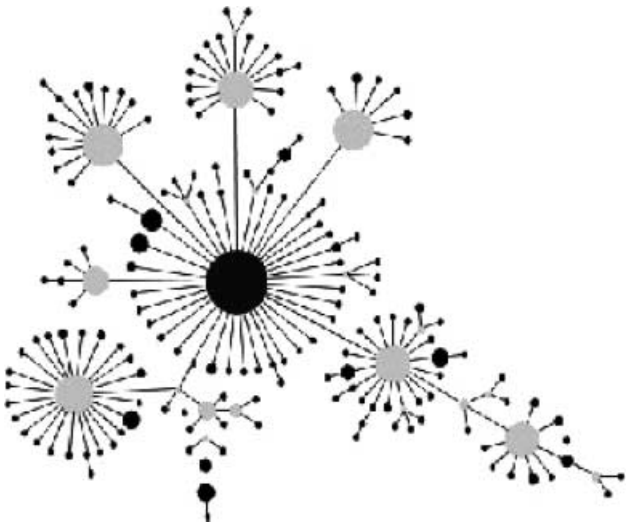
Objectives: The introduction of multilocus sequence typing (MLST) schemes for a number of pathogenic bacterial species has made it possible to draw rapid global comparisons between different disease-causing strains via the Internet. In order to fully exploit this resource, it is necessary to visualise the overall clonal structure of different species from entire MLST datasets of thousands of strains. Furthermore, a convenient means is required by which newly generated data can be compared against these datasets. Methods: The implementation of a new Java tool, eBURST, freely available on the www.mlst.net.
Results: The approach produces 'populations snapshots' showing all the major clonal lineages present within entire MLST data and also successfully untangles short-term patterns of descent within these lineages.



Conclusions: This approach provides a simple and intuitive means to visualise large MLST datasets and should help to standardise the nomenclature describing different clones within different pathogenic populations.

S338 Genetic portraits of methicillin-resistant *Staphylococcus aureus* clones in hospitals and in the community

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Oeiras, P

Objectives: In order to understand the emergence and spread of multidrug-resistant methicillin-resistant *Staphylococcus aureus* (MRSA), international surveillance systems such as the CEM/NET (Center for Molecular Epidemiology and International Network) initiative have been created. Over 3500 MRSA isolates collected between 1992 and 2003 in surveillance studies and outbreak investigations in hospitals located in Europe, Latin America, the USA, and Asia were characterised in order to create an inventory of MRSA clones in different regions of the world.
Methods: Isolates were initially characterised by pulsed-field gel electrophoresis, ClaI-mecA polymorphisms, and Tn554 insertion patterns and their identity was later re-analysed by spa typing, multilocus sequence typing (MLST), and SCCmec (staphylococcal cassette chromosome mec) typing.
Results: The large majority of the isolates belonged to a limited number of clones, namely the Iberian (ST247-SCCmecIA), Brazilian/Hungarian (ST239-III), New York/Japan (ST5-II), Paediatric (ST5-IV), and EMRSA-16 (ST36-II) clones. In contrast to these epidemic clones, minor clones (i.e. clones clearly dominant in some hospitals but not seen in others) and sporadic isolates (i.e. isolates recovered only from one or a few patients in a single hospital) were identified as well. MLST, spa typing, and SCCmec typing demonstrated extensive diversity among isolates belonging to minor clones or sporadic MRSA both in the genetic background and in the structure of the associated SCCmec elements. Nevertheless, the BURST (i.e. based upon related sequence types) algorithm grouped these isolates into restricted clonal complexes and predicted that most have evolved from pandemic MRSA clones. In addition, several of the sporadic MRSA resembled community-acquired MRSA isolates in properties that included a relatively limited multiresistance pattern, diversity of genetic backgrounds, faster growth rates, and a frequent association with SCCmec type IV.
Conclusions: There is evidence of the existence of only a few nosocomial epidemic MRSA clones spread worldwide. Some of the MRSA strains described as community-acquired may have originated in hospitals, while others seemed to have been transfered from the community to the hospital.

S339 Review of MDR tuberculosis in Russia

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Up to 1990 Russia had a recession of the main epidemiological indicators in tuberculosis. At active revealing the incidence rate was about 38 per 100 000. 700 TB laboratories gave an appropriate picture of drug resistant TB: H: 10–16%; R:6–8%; E: 4–6%; S: 14–18%. The problem to keep standard treatment regimens from 1992 to 1998, active migration, reduction of number of laboratories, generated resistant TB pool. The size and their influence on an epidemic situation in Russia could not be predicted. In 1996 the data came to: H: 24.6%; R: 18.7%, E: 19.5%, S: 34.3%. Since 1978 until 1998 14 million cultures and 1.17 million DST was performed. This data was not divided according to attributes for new cases and previously treated cases and the MDR estimation was not made. In 1999 the registration form of MDR was included in the federal annual statistics. The annual territorial reports on MDR varied from 1.3% (Ivanovo reg.) up to 47.1 % (North Caucasus) and reflected regional problems. To avoid the further spread of TB the new conception of TB National Programme was created. This programme includes detection, treatment, laboratory work, registration, quality control. For an estimation of resistant TB prevalence and recognition of approach to MDR treatment a few projects were developed. These projects were developed since 1998 at participation of WHO, CDC and other international agencies. The projects cover six regions with the population of more then 10 million people and include centralisation of TB service, the personnel training, external quality assessment of drug susceptibility test (DST), use of uniform laboratory and registration techniques, development of regiment treatments. The DST are performed by the method of absolute concentration. For an external quality assessment for region laboratories 50% of resistant and 25% of susceptible strains are quarterly directed for testing to Federal reference laboratory and independently CDC. The data was very close. So there is reliable data to estimate the increase of the MDR prevalence (from 1.4% in 1999 up to 7.7% in 2002 among new cases, Ivanovo region). In 2003 the project of MDR TB treatment started in Orel region. According previously data the good results were achieved in 72% of MDR cases. During the programme the territorial MDR indicators remained constant among new cases. The adverse effects during the treatment result in need to develop reliable DST for second line TB drugs and quality assessment.

S341 Microbial surveillance of diphtheria across Europe

A. Efstratiou, A. De Zoysa, J. White, N.S. Crowcroft on behalf of DIPNET

Global resurgence of diphtheria during the last 14 years, mainly within the Newly Independent States (NIS) of the former Soviet Union and South East Asia, has stimulated renewed interest in this 'vaccine preventable disease'. A major goal for many international has been to improve surveillance for early detection of emerging and re-emerging diseases by the establishment of laboratory and disease specific networks. These epidemics have major implications for Europe and its associated countries, where there are many immigrants from the NIS and Eastern Europe. Monitoring the spread of diphtheria and related infections caused by toxigenic strains of *Corynebacterium diphtheriae* and *C. ulcerans* has been the basis of several European Commission funded programmes during 1998–2004. These programmes along with effective liaison with WHO EURO have established a network of Diphtheria Reference Centres across Europe and have improved communication and exchange of information between countries. Initially, the European Laboratory Working Group on Diphtheria, ELWGD, was established at the request of WHO EURO in 1993, in response to the epidemics in the NIS, which comprised the key microbiologists responsible for diphtheria in each country. Participation within the ELWGD grew since 1993, from 10 to 40 countries globally by 2002. The effective collaboration was significantly enhanced by the participation of the key epidemiologists from each country in 2000, to establish DIPNET, the Diphtheria Surveillance Network, now officially recognised by the EU as a disease specific network. The collaborations have enhanced laboratory diagnostics considerably and have also established a definitive genotyping scheme for the identification of epidemic and nonepidemic clones. Ribotyping was identified by the ELWGD as the most discriminatory method for tracking the international dissemination of diphtheria, particularly useful in the NIS epidemics for identifying the main epidemic genotype, 'Sankt Petersburg' and also for identifying the geographic origin of strains globally. More than 70 ribotypes have been recognised thus far to build and establish a genotype database. *C. diphtheriae* appears to be a species with significant genetic diversity with the potential for changes that could facilitate the appearance of large-scale epidemics, thus highlighting the importance of monitoring the molecular epidemiology of strains globally.

Auditing antimicrobial prescriptions in Europe (Symposium arranged with ESGAP)

S342 Audit Project Odense for General Practice

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Background: For the past 10 years, Audit Project Odense (APO) has been performing audits in general practice in the Nordic countries. Audits on the diagnosis and treatment of respiratory tract infections (RTIs) have been the most successful.
Objective: The aim of the present study was to maintain, and possibly improve, a restrictive antibiotic policy for RTIs via audit of general practitioners (GPs), feed back of results and group discussion.
Methods: In November to December 2001, an audit on diagnosis and treatment of RTIs was carried out in Denmark, Norway, Sweden, Finland and the Faroe Islands. In Denmark, 366 GPs took part in the project. Audit data were registered by the GPs themselves according to the APO method (http://www.Apo-Danmark.dk). The well-validated standard APO registration chart was used. These data were supplemented with data from the central prescription register of the County of Roskilde. Data were analysed at the APO institute and reports were sent to all participating GPs. Follow-up meetings were arranged, where the results were compared and discussed with colleagues and local guidelines could be elaborated. A second registration was carried out 1 year later.
Results: Important quality problems, i.e. high-level and inappropriate consumption of macrolides, diverging use of diagnostic tools and great variation between GPs on a number of other parameters such as the frequency of antibiotic prescribing, the frequency of macrolide prescribing and the ratio narrow-/broad-spectrum penicillins, were identified in all countries. In Denmark, the frequency of macrolide prescription varied from 11 to 37% and the ratio narrow-spectrum penicillin/broad-spectrum penicillin use varied from 0.9 to 6.8, depending on the GP. Self-registered data from the second registration showed no essential

changes in Denmark. However, central register data showed a decline in macrolide consumption.
Conclusion: The APO method has proven a useful instrument in maintaining a rational antibiotic policy among GPs in Denmark and other Nordic countries. Moreover, our experience shows that it is possible to improve the prescription habits of GPs using the APO method. The combination of self-registered data and central register data seems to represent an ideal basis for discussion. Indicators, calculated on the basis of register data, can be followed regularly. Information on APO can be found at: http://www.Apo-Danmark.dk E-mail: apo@health.sdu.dk, syks@ra.dk.

S343 Antibiotic prescribing in the primary paediatric care in Central Eastern Europe – common history with different approaches

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ESAC project shows important differences of antibiotic consumption in ambulatory care in CEE countries. Antibiotic consumption in the Czech Republic (CZ) and Hungary (HU) is about 20 DID, in Slovakia (SK) and Poland (PL) is about 25 DID. While the consumption in CZ, HU and SK is stable, there is ongoing increase in PL. Prevalence of resistance is also different, occurrence of penicillin-resistant pneumococci is high in SK and HU, low rates are in CZ and PL. Growing resistance of *S. pyogenes* to macrolides, especially in primary paediatric care, indicates changes in prescribing. ESAC consumption data describe current, relatively stable situation, but the most important changes in antibiotic prescribing were happened during early 1990s, when the healthcare system was totally transformed in all of former members of Soviet block. As example, in CZ, there was observed rapid increase of total consumption (14–19 DID) including deep changes of its structure (decrease of penicillins, increase of broad spectrum drugs). Changes in consumption were followed with growing resistance. These countries are under strong marketing pressure from the pharmaceutical industry, without experience on how to overcome it. It is extremely important to support the implementation of independent and effective intervention methods for improving antibiotic prescribing in the region. Project on auditing antibiotic prescribing in primary paediatric care was established in the late 1990s in the Czech Republic and Slovakia. The same protocol and methodology was applied for repeated multicentric studies, performed in several regions. Although both countries have common history in the same state for more than 70 years, and transformation of healthcare system was very similar, antibiotic prescribing practices and also resistance rates are extremely different. Antibiotic prescribing for acute respiratory illness is twice more frequent in SK, prescribing for common cold is extremely rare in CZ, while in SK is common. Audits successfully identified proportion and reasons of inappropriate prescriptions, when acute bronchitis is the most important for antibiotic misuse. Although CZ situation looks better, the proportion of inadequate prescribing is 50% as a minimum. The most of repeatedly audited GPs significantly improved their practice during the time. Based on the 5 years of experiences, there is good opportunity to extend auditing as a routine tool for interventions.

S344 Antibiotic prophylaxis in surgery: the Dutch experience

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Several factors are believed responsible in helping facilitate the low rates of bacterial antibiotic resistance in the Netherlands as compared with other European countries and the USA. These factors include, the influential position of the medical microbiology and infectious diseases profession, the establishment of elaborate infection control programs, and cultural influences (including the traditional prudent use of antimicrobials). Furthermore, in a recent survey, the majority of Dutch physicians reported no reluctance to clinical guidelines. Evidence-based guidelines on surgical prophylaxis have been developed to improve the quality of care and to reduce inappropriate prescribing in many countries. Various authors still report frequent overuse. The Dutch Working Party on Antibiotic Policy (SWAB), issued national guidelines for surgical prophylaxis in the year 2000, and these have been used as a framework for the development of local policies. The goal of these guidelines (besides reducing the incidence of surgical site infections), was to limit the spectrum of the antibiotics (based upon microbiology data from the Netherlands), promoting single dose administration and optimising the timing to within 30 min preoperative. The Surgical Prophylaxis and Surveillance Study (Chirurgische Profylaxe en Surveillance, CHIPS) investigated the implementation of this SWAB guideline in a prospective intervention study involving 13 Dutch hospitals. The intervention consisisted of feedback, educational and logistic support. Process outcome was documented both several months before and after intervention, by recording the choice, duration, dose and timing of antibiotic prophylaxis. SSI were monitored as the patient outcome indicator, with the Dutch national surveillance network (PREZIES) providing the SSI data. The study succeeded in increasing the use of first generation cephalosporins as a single dose and in improving the timing of prophylaxis, while maintaining efficacy in terms of preventing SSI. At present, further national projects are ongoing and are especially targeted towards the timing of prophylaxis (a factor particularly sensitive to organisational constraints). The guideline program of SWAB, the PREZIES network, the CHIPS study and the ongoing quality improvement program are supported by the Dutch government. In this presentation, quality indicators of process and outcome of surgical prophylaxis will be compared with international data from the literature.

S345 Antibiotic prescribing practices and policies in intensive care units

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Multidrug-resistant microorganisms are commonly found in intensive care units (ICUs), which are now recognised as 'hot zones' for antimicrobial resistance in hospitals. However, much less is known about antibiotic prescribing practices in ICUs. Aggregated pharmacy data on antimicrobials dispensed to ICUs are generally used for this purpose. Data from ICU surveillance networks such as the Swedish IVA-STRAMA and the German SARI, as well as data from Denmark and from studies in single hospitals, show that ICU antimicrobial use varied from 490 to 3456 WHO Defined Daily Dose (DDD)/1000 patient-days. This means that on average on a given day, most ICU patients receive one antimicrobial, if not a combination of antimicrobials. This also means that the ecological pressure due to antimicrobials is generally higher in ICUs than in most hospitals (around 40–80 DDD/100 patient-days or 400–800 DDD/1000 patient-days) and much higher than in ambulatory care (around 10–40 DDD/1000 inhabitant-days, depending on country). When patient-level data are available, one can calculate the proportion of patients exposed to antimicrobials during ICU stay. From various studies, it can be estimated at 50–80%. Such patient data also allow the measurement of the number of daily antimicrobial treatments as in the EC-funded 'European Strategy for Antibiotic Prophylaxis' (ESAP) project. Certain policies seem to be associated with high/low antimicrobial prescribing in ICUs. The ESAP project showed that two ICUs that routinely used selective decontamination of the digestive tract (SDD) reported 3753 and 4794 daily antimicrobial treatments/1000-patient days, respectively, which was four to five times the median ecological pressure of 928 daily antimicrobial treatments/1000-patient days observed in 21 other European ICUs that did not use SDD. Among these, having a list of antimicrobials subject to restricted use and reporting excellent communication between senior and junior doctors were independent factors associated with low use. The EC-funded 'Antibiotic Resistance Prevention

And Control' (ARPAC) project should soon provide more recent and detailed data on antimicrobial use and policies in European ICUs. These will form the basis for the implementation of interventions. However, additional efforts are still needed to better understand and improve the quality of antimicrobial prescriptions in ICUs. This may be addressed by repeated audits of prescribing practices.

Antibiotic resistance: from ESBL to fluoroquinolones

O346 EARSS report on increasing fluoroquinolone resistance among *Escherichia coli* in Europe

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Objectives: We explored proportions and trends of fluoroquinolone-resistant *E. coli* isolated from blood-cultures reported to the European Antimicrobial Resistance Surveillance System (EARSS) by participating laboratories.
Methods: Participating laboratories carry out routine antimicrobial susceptibility testing (AST) for invasive *E. coli* isolates. Fluoroquinolone resistance in *E. coli* is defined as a minimum inhibitory concentration (MIC) of >4 mg/L to either ofloxacin or ciprofloxacin. 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: From January 2001 until October 2003, 24 countries reported AST results for 38 370 *E. coli* isolates. A significant increase in resistance to fluoroquinolones, from 8% (666 of 8411 isolates) in 2001 to 10% (479 of 4684 isolates) in 2003 was observed in laboratories that participated all 3 years. Resistance ranged from 3% in Ireland (155 isolates) to 21% in Italy (617 isolates). Between 2001 and 2003, resistance increased in 15 of the 24 countries. For eight countries this increase was significant (two-sided $P < 0.05$). No country showed a significant decrease in resistance proportion.
Conclusions: Our data suggest a common trend of expanding fluoroquinolone resistance in all parts of Europe.

O347 First detection of the transferable quinolone resistance determinant in clinical *Providencia stuartii* strains in Egypt

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Objectives: Quinolones are potent antibiotics that target the bacterial DNA gyrase and topoisomerase IV. The recently described plasmid-encoded Qnr protein protects both enzymes from quinolone inhibition which leads to low-level resistance and is therefore able to facilitate the selection of high-level resistance. The qnr determinant has been found in *E. coli* and *K. pneumoniae* strains in the USA and in *E. coli* strains in Shanghai, China. However, studies including strains from diverse geographical origins did not result in further findings. Here we describe the detection of the qnr resistance determinant in clinical strains isolated in Egypt.
Methods: Strains used in this study were isolated in a burn unit in a university hospital in Cairo, Egypt between April and October 2001. MIC values were determined using a microdilution method according to the NCCLS and E-tests. Strains with ciprofloxacin MIC values of 0.25 mg/L or higher were screened for possession of qnr using primers binding at the 5′ and 3′ end of the gene. These included the following species: *E. coli* ($n = 4$), *K. pneumoniae* ($n = 4$), *E. cloacae* ($n = 1$), *S. marcescens* ($n = 2$), *P. rettgeri* ($n = 4$), *P. stuartii* ($n = 4$), *M. morganii* ($n = 1$) and *P. mirabilis* ($n = 10$). One qnr PCR product was sequenced. Epidemiological relationship was examined using RAPD-PCR methods. Conjugation experiments were performed using *E. coli* W3100 RifampicinR as the recipient.
Results: Screening for qnr revealed the resistance determinant in four *P. stuartii* isolates RAPD-PCR indicated that two of the *P. stuartii* strains were clonally related. The three unrelated isolates show intermediate quinolone susceptibility and resistance (Table 1). Sequencing of one PCR product derived from *P. stuartii* Ps125 showed 100% identity with the published qnr sequence found on *E. coli* plasmids. The qnr determinant of all strains was transferable to *E. coli* W3110 RifR. All transconjugants had the same susceptibility phenotype and showed elevated quinolone MIC values compared with the recipient (Table 1).

Table 1. MICs (mg/L) of *P. stuartii*(Ps) strains and their derived transconjugants(TC)

Antimicrobial Agent	Ps121	Ps122	Ps125	TCPs121 TCPs122 TCPs125	*E. coli* W3110 RifR
Ciprofloxacin	2	0.5	4	0.06	0.004
Norfloxacin	2	0.5	16	0.25	<0.016
Levofloxacin	n.d	n.d	n.d	0.064	0.004
Moxifloxacin	16	2	8	0.125	0.03
Gatifloxacin	8	1	8	0.06	<0.016

Conclusion: Until now, qnr has only been found in clinical *E. coli* and *K. pneumoniae* strains. However, it had been shown that plasmids carrying the qnr gene have a broad host range. The detection of transferable quinolone resistance in *P. stuartii* shows the extension of the host spectrum of qnr plasmids in the clinical setting. Though qnr genes are still rarely detected, finding of qnr in Egypt points to a wide geographic distribution of this resistance determinant.

O348 Fluoroquinolone consumption and fluoroquinolone resistance in haematology–oncology patients – ecological analysis in two university hospitals, 1999–2002

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Objectives: The MABUSE (Medical AntiBiotic Use Surveillance & Evaluation) network, a German cooperative group of infectious disease specialists, WIdO, the SARI programme and hospital pharmacists, collects antibiotic consumption data in ambulatory and hospital care (HC) settings. In a HC antibiotic consumption study of eight university hospital medical services, we identified two services with extremely low and extremely high consumption of fluoroquinolones in haematology–oncology departments. We compared fluoroquinolone consumption data in these services with fluoroquinolone resistance rates among *Escherichia coli* (EC), *Pseudomonas aeruginosa* (PA), and staphylococcal isolates from patients admitted to the services.

Methods: Consumption data were expressed as DDD per 100 patient days (DDD/100) using the WHO/ATC definition. An alternative daily dose definition using local guidelines was used and expressed as prescribed daily doses per 100 patient days (PDD/100). *In vitro* susceptibility to fluoroquinolones (ciprofloxacin and/or levofloxacin) of bloodstream isolates of EC and coagulase-negative staphylococci (CNS), and of PA and *S. aureus* (SA) clinical isolates from other body sites was performed according to DIN (Deutsches Institut für Normung) guidelines and breakpoints using microbroth dilution or agar diffusion tests.
Results and Conclusions: Hospital A haematology–oncology services showed a fluoroquinolone use density of 75 DDD/100 (4-year average, 1999–2002, range, 53.2–92.8) which was significantly higher than in hospital B (4-year average, 12 DDD/100; range, 9–13.2). Applying the alternative PDD definition, fluoroquinolone use in hospital A was still much higher than in hospital B (4-year averages, 37.9 vs. 10.7 PDD/100), while total consumption of antibiotics was similar (81.9 vs. 81.4 PDD/100). Rates of *in vitro* resistance to fluoroquinolones were high in hospital A haematology–oncology service isolates of EC (range, 64–79%) compared with rates for hospital B (range, 8–11%), and correlated well with the consumption data. The correlation between use and resistance was less clear for trimethoprim-sulphamethoxazole resistance of EC and for fluoroquinolone resistance of staphylococci and PA. Fluoroquinolone resistance among coagulase-negative staphylococci was surprisingly high in both hospital A and B haematology–oncology services (ranges, hospital A, 68–97%, hospital B, 70–79%).

O349 Genetic analysis of emerging fluoroquinolone-resistant *Escherichia coli* in Indonesia

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Objectives: In a recent population-based survey of 4000 people in two cities in Indonesia (Surabaya and Semarang) fluoroquinolone-resistant *Escherichia coli* (FQREC) were found to be emerging. FQREC were prevalent in the faecal flora of patients at times of admission and discharge from hospital, but not among healthy relatives nor patients visiting primary healthcare centres. We studied the phylogenetic background and clonal relatedness of FQREC and compared them with that of fluoroquinolone sensitive strains (FQSEC) isolated in the same population.
Methods: 196 FQREC and 200 FQSEC strains were assigned to one of four phylogenetic groups (A, B1, D, B2) using multiplex PCR for the presence of determinant genes *chuA*, *yjaA* and *TspE4.C2*. Clonality was determined by PCR fingerprinting using ERIC primers. Mutation rates were assessed using rifampicin-containing media (*Antimicrob Agents Chemother* 47, p. 3222).
Results: The distributions of FQREC and FQSEC across the phylogenetic groups A (57% vs. 52%), B1 (23% vs. 30%), D (20% vs. 11%) and B2 (1% vs. 7%) differed significantly ($P = 0.001$). The mutation rates of the strains varied widely, but those of FQREC strains were generally higher compared with the mutation rates observed among FQSEC strains ($P < 0.0001$). Phylogenetic grouping of the strains of the two cities was highly similar. Clonal analysis of 196 FQREC yielded 110 different genotypes. Sharing of genotypes was observed among strains isolated from patients at the time of discharge from the same hospital, indicating nosocomial spread of FQREC in each of the two hospitals. The number of patients sharing the same FQREC genotype varied from 2–8. Transmission of FQREC was found in departments of Internal Medicine, General Surgery and Gynaecology.
Conclusions: The emergence of FQREC in Indonesia predominantly involves the relatively low virulence phylogenetic groups A and B1, but FQREC has also emerged in higher virulence groups D and B2. The emergence of FQREC in Indonesia is so far largely restricted to those groups of patients that require and are admitted to hospital care where selection and spread of FQREC can be documented.

O350 Outpatient use of fluoroquinolones in Europe with focus on respiratory tract infections

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Objectives: We investigated geographical differences, temporal trends and seasonal fluctuations of the outpatient use of fluoroquinolones (FQ) in Europe within the ESAC project, funded by DG SANCO, using the ATC/DDD method (WHO, version 2003). We focused on the new FQ, levofloxacin (LEV) and moxifloxacin (MOX), which were marketed for the treatment of respiratory tract infections (RTI), particularly due to penicillin and/or macrolide resistant *Streptococcus pneumoniae*.
Methods: We assessed outpatient FQ (J01M ATC group) use from 1997 to 2002 from 24 countries, and use data were expressed in DDD per 1000 inhabitants per day (DID). Seasonal data were available from 19 countries.
Results: Total outpatient FQ use in 2002 varied with a factor of 21.2 between countries with the highest and the lowest consumption (3.76 DID in Italy vs. 0.17 DID in Denmark). Norfloxacin still represented the most widely prescribed FQ in 2002 in Croatia (89.0% of the J01M group), Czech Republic (46.9%), Sweden (44.4%), Slovenia (46.0%), Latvia (43.6%), Hungary (33.8%) and France (35.9%). In the 17 other countries, ciprofloxacin (CIP) was the most widely prescribed FQ in 2002, except in Italy and Belgium (LEV most prescribed FQ) and in Slovakia (ofloxacin most prescribed FQ). In all but one country (Portugal, PT) we found no seasonal variation of CIP, which is consistent with it being administered as therapy of adult RTI. Only in Belgium (BE) and PT, and to a lesser extent in Austria, LEV showed seasonal fluctuations, with peaks during the winter season (peak of 1.64 DID and 0.97 DID in the winter of 2002, in BE and PT, respectively). Although consumption is lower and only seen from the second half of 2000 onwards, the same seasonal trend was observed with MOX, with the highest winter peaks in BE.
Conclusions: A low seasonal fluctuation of the earlier FQ, such as CIP, may be a good marker of restrained antibiotic prescription. The introduction of LEV and MOX was very successful in BE and PT. The Belgian data are surprising because all guidelines clearly state that these drugs are not first-line therapy for adult RTI. Sounding the alarm about the peril of rising antimicrobial resistance may be inadvertently promoting inappropriate use of these new FQ, which will inevitably lead to emergence of resistance.

O351 Extended-spectrum beta-lactamases from the community and hospital environments in Portugal: dissemination of TEM-24 *E. aerogenes* European epidemic clone and emergence of CTX-M enzymes

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Objectives: To evaluate the prevalence and epidemiology of ESBL-producing Enterobacteriaceae from the community and hospital setting in Portugal.
Methods: Twenty-eight raw poultry products, two sewage, 25 swine and 85 healthy volunteers faecal samples (1999–2003) were cultured in both MacConkey agar and MacConkey broth with or without ceftazidime (1 mg/L) and cefotaxime (1 mg/L). One colony per morphology was selected for posterior studies. Sixty-three recent clinical isolates from three hospitals of the area under study (18 *K. pneumoniae*, one *K. oxytoca*, 13 *E. coli*, four *E. aerogenes*, five *E. cloacae*, 13 *S. marcescens*, six *P. mirabilis*, one *M. morganii*, one *C. freundii* and one *P. stuartii*) were included. All isolates were screened for ESBL by the double disk synergy test. ESBLs were characterised by IEF, PCR for blaTEM, blaSHV, blaCTX-M-9 and blaCTX-M-10, and sequencing. Susceptibility to non-beta-lactam antibiotics was performed by the disk diffusion method. Clonality was searched by PFGE.
Results: ESBL producers were obtained from 10 poultry (12 *E. coli* and four *K. pneumoniae* isolates), two sewage (two *E. coli*) and

three healthy volunteers faecal samples (three *E. coli*), but not in those from swine samples. Beta-lactamase characterisation revealed the presence of TEM (pI = 5.4, 5.6, 5.9) and SHV (pI = 7.6) ESBL-types, both in nonclinical and clinical isolates. A CTX-M-type enzyme, CTX-M-14, was detected for the first time in our country in an *E. coli* isolate from a healthy volunteer. blaTEM-24 was identified in three *E. aerogenes* clinical isolates which by PFGE revealed the same pattern of the *E. aerogenes* epidemic clone reported in French, Belgium and Spanish hospitals. Nonsusceptibility rates of ESBL-producing organisms of clinical and nonclinical origin were as follows: gentamicin (42–17%), streptomycin (85–75%), sulphonamides (84–92%), trimethoprim (83–75%), tetracycline (88–83%), chloramphenicol (56–33%), nalidixic acid (85–75%) and ciprofloxacin (70–47%).
Conclusion: ESBLs were found in Enterobacteriaceae from Portuguese hospitals, poultry, sewage and healthy volunteers samples. An european epidemic clone with TEM-24 was detected in isolates from different hospitals of our country, but interestingly, the presence of the worldwide-distributed CTX-M ESBLs was only demonstrated in the community. This might be related to local differences in the genetic structure of bacterial populations from hospital and community environments.

O352 Isolation of extended-spectrum beta-lactamase producing Enterobacteriaceae from community-based patients in the United Kingdom

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Objectives: Extended-spectrum beta-lactams are commonly included in empirical antibiotic regimens for the treatment of Gram-negative infections. The emergence of extended spectrum beta-lactamase (ESBL)-producing bacteria poses a serious threat to the continued use of this family of antibiotics. To date, ESBL-producing bacteria have been reported worldwide, mainly in hospital settings. The aim of this study is to ascertain the level of ESBL-producing bacteria in clinical samples submitted from patients outside of hospital.
Methods: Over a 3-month period, we saved all cefradine-resistant Enterobacteriaceae collected from urine samples submitted to our laboratory for microbiological analysis. Isolates were provisionally identified by their colonial appearances on CLED medium and confirmed using the API 20E system. Antibiotic susceptibility to a range of antibiotics was determined using Stoke's disk diffusion method. All the isolates were screened for ESBL production using the standard Disc Synergy Testing (DST) and the commercially available MAST DD test. Polymerase chain reaction (PCR) using specific primers was used to screen for the presence of blaSHV and blaTEM in the isolates scoring positive for ESBL production. Nucleotide sequence analysis was used to determine the identity of the resistance determinants.
Results: Eighty-four isolates from 78 patients were obtained during the study period. Forty-two (54%) were community-based patients. ESBL production was detected in 16 isolates from 10 patients (13%): nine *Klebsiella* species, five *Enterobacter* species, one *Escherichia coli* and one *Citrobacter freundii*. Three of the ESBL+ patients were community-based, only one of whom had recently been discharged from hospital. PCR showed that eight isolates harboured blaSHV while two isolates harboured blaTEM. Nucleotide sequence analysis of an internal fragment of the blaSHV showed the isolates to produce an SHV-2 like ESBL (five isolates). Identity of the ESBLs in the remaining isolates has not been determined yet.
Conclusion: This is the first report describing the presence of extended-spectrum beta-lactamases in UK community-based patients. This highlights the continuing global emergence of these clinically important enzymes and the importance of screening for their presence not only in hospitalised patients, but in the community as well.

O353 Characterisation of a new integron carrying a metallo-beta-lactamase and a carbenicillinase from *Pseudomonas aeruginosa*

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Objectives: An increasing prevalence of carbapenem resistance mediated by acquired metallo-beta-lactamase genes (*blaIMP* and *blaVIM*), inserted in integrons, is being reported, particularly for *Pseudomonas aeruginosa* clinical isolates. Here, we report the genetic characterisation of a new integron carrying a carbapenem resistance determinant, *blaVIM-2*, from a *P. aeruginosa* clinical isolate.
Methods: A clinical imipenem-resistant *P. aeruginosa* strain was isolated in a Portuguese hospital from a blood culture. MICs for beta-lactam antibiotics (including carbapenems) were determined by the Etest method. IEF was also performed in crude extracts. Susceptibility to aminoglycosides was assessed by the disk diffusion method. The presence of metallo-beta-lactamases was investigated, using a bioassay and a PCR multiplex for *blaVIM* and *blaIMP* genes. Class 1 integrons were screened with sets of primers specific for 5'CS and 3'CS. The obtained amplicon was cloned in pPCR-ScriptTM Cam SK(+) plasmid vector and expressed in *Epicurian coli* XL10-Gold. Characterisation of integron containing the metallo-enzyme gene was possible through sequencing.
Results: The *P. aeruginosa* isolate was resistant to most beta-lactams, including imipenem (>32 mg/L), meropenem (>32 mg/L), ceftazidime (32 mg/L), but remained susceptible to aztreonam (8 mg/L). The isolate was also resistant to tobramycin, gentamicin, amikacin, netilmicin and ciprofloxacin. A positive bioassay was observed, suggesting a metallo-beta-lactamase and preliminary PCR-based experiments detected the metallo-beta-lactamase *blaVIM-2*. Two beta-lactamases of pI 5.3 and 5.7 were observed in crude extracts by IEF. Sequencing of the cloned PCR amplicon, containing the gene cassette *blaVIM-2*, revealed the structure of a new class 1 integron. In fact, assembly of sequence data, showed *blaVIM-2* and *blaP1b*, two beta-lactamases that have never been associated in the same integron before. The integron also carried two aminoglycoside resistance genes.
Conclusions: To our knowledge, this is the first description of an integron that contains a metallo-beta-lactamase associated to another beta-lactamase.

O354 Epidemiology and clinical features of bacteraemia due to extended-spectrum betalactamase-producing *Escherichia coli*

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Objectives: The rate of bacteraemia because of extended-spectrum beta-lactamase-producing *Escherichia coli* (ESBLEC) seems to be increasing, but specific information about this infection is scarce as they are usually described together with episodes caused by ESBL-producing *Klebseilla pneumoniae* (ESBLKP). We describe the clinical features and the epidemiology of a series of bacteraemia because of ESBLEC.
Methods: We included all episodes of bacteraemia because of ESBLEC in out centre from January 2001 to June 2003. ESBL production and antimicrobial susceptibility were tested by microdilution following NCCLS guidelines. Relatedness of the isolates was performed by REP-PCR. ESBL were preliminary characterised by isoelectric focusing and PCR. The sequencing of the genes encoding the ESBLs is in progress.
Results: Nineteen episodes were included. The distribution of the episodes showed an increasing trend throughout the study period, from 3.9% of the episodes of bacteraemia because of *E. coli* in 2001 to 8% in 2003. The mean age of the patients was 70 years, 74% were male, and all of them had comorbidities. Ten episoded

were community-acquired (eight could be considered health-care related) and nine were nosocomially-acquired (none of them were in the ICU). Three patients were neutropenic, and 14 had recently received antimicrobials (seven fluorquinolones). The main sources of bacteraemia were the urinary tract (48%) and the biliary tract (26%); it was unknown in 16%. Sixteen per cent had severe sepsis and 5% septic shock. Fifty-three per cent of the isolates were susceptible to amoxicillin/clavulanic acid, 95% to piperacillin/tazobactam, 100% to imipenem, 32% to ciprofloxacin, and 84% to gentamicin. Empirical antimicrobial treatment was considered appropriate according to *in vitro* susceptibility data only in 37% of the episodes (10 patients received extended spectrum cephalosporins). One patient died, and it was related to the infection. Only the isolated from three patients were clonally related; 84% of the isolates produced CTX-M type ESBL.
Conclusion: Bacteraemia caused by ESBLEC is an emergent infection that affects predisposed patients, most of whom had received antimicrobials. About half of the cases were community-acquired, although most of them are health care-associated. Most of the isolates were not clonally related and produced an CTX-M type ESBL. These data suggest that the epidemiology of bacteraemia due to ESBLEC has relevent differences with ESBLKP.

O355 The first report of a metallo-beta-lactamase-producing *Proteus mirabilis* clinical isolate from Greece

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Objectives: *Proteus. mirabilis* is the second most common cause of urinary tract infections and also an important cause of nosocomial infections. Class B metallo-beta-lactamases (MBLs) have recently been reported in several strains of Gram-negative bacilli but not yet in *P. mirabilis*. In 2003, a *P. mirabilis* strain with a reduced susceptibility to imipenem was isolated in a tertiary care hospital in Athens (Greece). The strain demonstrated a positive EDTA-disc synergy test and it was studied for the presence of a MBL gene.
Methods: *Proteus mirabilis* was isolated from the cerebrospinal fluid of a patient suffering from meningitis secondary to craniocerebral trauma. Susceptibility testing was performed by the disk diffusion technique and MICs were determined by the broth microdilution method, according to the NCCLS guidelines (M7-A5, Vol. 20, No. 2, 2000). EDTA-disc synergy test, was used to screen for MBL production. Beta-lactamases were detected by isoelectric focusing (IEF) and the MBL gene was identified by PCR with the following set of primers: VIM-F (5′-ATGGTGTTTGGTCGCATATC-3′) and VIM-B (5′-TGGGCCATTCAGCCAGATC-3′). Sequencing of cloned PCR products was performed by MWG – The Genomic Company.
Results: The *P. mirabilis* isolate was resistant to ampicillin, cephalothin and cefuroxime, had reduced susceptibility to ceftazidime and cefepime (MIC 8 and 4 mg/L, respectively) and was fully susceptible to aztreonam (0.25 mg/L). MICs of imipenem and meropenem were 2 and 0.25 mg/L, respectively. EDTA-disc synergy test was positive suggesting the presence of a MBL. IEF identified a beta-lactamase with a pI of approximately 5.4. Sequencing of the cloned PCR product identified the blaVIM-1.
Conclusions: To the best of our knowledge, this is the first time that a MBL gene has been detected in *P. mirabilis*. The spread of MBLs in Enterobacteriaceae has enormous therapeutic implications. Routine susceptibility testing cannot be used to predict the presence of MBLs, and laboratories must be prepared to screen MBL-producing isolates in order to guide the prompt implementation of infection control measures and the proper treatment of the patient.

Hepatitis

O356 HCV core, F, NS3, NS4B and NS5A are the major immunogenic proteins in humoral immunity in chronic HCV infection

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Hepatitis C virus (HCV) genome encodes for three structural (core, E1 and E2) and six nonstructural (NS2, NS3, NS4A, NS4B, NS5A and NS5B) as well as a recently identified F protein produced from an alternative reading frame from the core region. In order to study humoral immune responses against individual HCV proteins they were produced by baculovirus of *Escherichia coli* expression. The proteins were purified by preparative SDS-PAGE and antibody responses against different HCV proteins were analysed by quantitative Western blotting. The study material included serum specimens from 68 individuals chronically infected with HCV. All serum specimens were HCV RNA positive, and 21, 20, 23 and four individuals were of HCV genotypes 1, 2, 3 or 4, respectively. The core, F, NS3, NS4B and NS5A were the major antigenic proteins, and 97, 94, 68, 85 and 53% of the individuals had antibodies against these proteins, respectively. Lower rate of antibody positivity was observed also against E1, E2, NS4A and NS5B (4–28%) proteins, whereas NS2 protein appeared to be completely nonantigenic. As analysed by Western blotting the mean antibody levels were 4–30-fold higher against the core protein (mean titre 1:35 000) as compared with the other HCV proteins. Clearly detectable antibody levels were also observed against F (mean titre 1:1500), NS3 (1:4000), NS4B (1:9000) and NS5A (1:4500) proteins. No significant differences in antibody levels were seen in infections caused by different HCV genotypes. Follow-up of serum specimens from five individuals during a period of 2 years revealed that the antibody specificity and their levels remained remarkably constant in each individual. Our results indicate that baculo/ *E. coli*-produced HCV proteins are well suitable for diagnostic purposes and there is a tremendous variability in individual anti-HCV antibody levels and protein specificity.

O357 Comparative analysis of intra-hepatic mRNA levels for IFNs and IFN-related genes in HCV mono-infected and in HCV/HIV co-infected patients

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Objectives: Hepatitis C virus (HCV) infection runs a more rapid and severe course of liver disease in HIV-1 co-infected patients and treatment of HCV is at present a major challenge in these individuals. In a previous work we showed that mRNA levels for IFNs and for some IFN related genes were altered in HCV mono-infected subjects, as compared with nonalcoholic steato-hepatitis (NASH) patients. In this study we analysed mRNA levels for IFN-alpha, IFN-gamma, IFNAR-1 and PKR in liver biopsies of HCV/HIV co-infected patients to compare their levels with those found in HCV-monoinfected subjects.
Methods: To this aim liver biopsies from 20 HIV/HCV co-infected patients and from 24 HCV-infected patients, similar for demographic features, HCV viral load and genotypes, as well as for liver histology, were analysed. Total RNA was extracted from liver biopsy, and a limiting dilution RT-PCR was performed by using primers specific for IFN-alpha, IFN-gamma, IFNAR-1 and PKR.

Results: In HCV/HIV co-infected patients intra-hepatic IFN-alpha mRNA levels were up-regulated as compared with HCV monoinfected patients, whereas IFN-gamma, IFNAR-1 and PKR were profoundly down regulated, as in all biopsies from co-infected patients they were under detectable levels. We observed a positive correlation between IFN-alpha mRNA levels and HIV-RNA viral load ($r = 0.526$, $P = 0.018$, in Spearman rank sum test). In co-infected patients, similar mRNA levels for IFN-alpha were found in patients with HCV viraemia higher or lower than 500 000 IU/mL, and they were not related to ALT levels or CD4 cell counts. Furthermore, no different IFN-alpha mRNA levels were observed in co-infected patients with absent/mild vs. moderate/severe fibrosis. This is at variance with monoinfected patients, were IFN-alpha mRNA levels were higher in patients with lower extent of fibrosis.
Discussion: The presence of up-regulated IFN-alpha mRNA, together with a parallel absence of mRNA for IFNAR-1 in liver biopsies of HIV-HCV-co-infected patients indicate that, in spite of a strong activation of IFN-alpha expression, driven presumably by HIV, there is an impaired ability to respond to IFN-alpha action, because of the lack of expression of its receptor. This is also supported by the virtual absence of mRNA for the main IFN-alpha effector protein (PKR). These results may have important implications regarding the pathogenesis of the liver damage and the therapeutic regimens to be used in co-infected patients.

O358 Sustained response to interferon-ribavirin combination therapy in chronic hepatitis C predicted by a model of viral dynamics using both HCV-RNA and alanine amino transferase

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Objectives: Serum hepatitis C virus (HCV) RNA decreases quickly in the first 48 h of alpha interferon (IFN) therapy (1st phase) and slowly thereafter (2nd phase). Standard mathematical models found a correlation between the clearance of infected cells (2nd phase) and the response to IFN, however do not predict whether the response will be maintained. Aim of this work was to identify new parameters that can predict the sustained response early during treatment.
Methods: We developed a new model in which the rate of infected cells clearance is computed by the alanine-aminotransferase (ALT) decline during the first month of therapy and, thereafter, it diminishes according to the infected cell number. The model computes and fits HCV-RNA and ALT variations with the values measured during the first month of therapy (days: 0, 2, 4 or 5, 7, 14, 21, 28) and calculates the number of infected cells. We analysed 28 of 31 chronic hepatitis C patients consecutively treated with IFN alfa 2b 3–5 MU, with or without Ribavirin, for 6 or 12 months depending on HCV genotype.
Results: The percentage of infected hepatocytes computed at baseline was consistent with that reported by *in situ* studies and lower in seven sustained responders (mean: 11.7%, range: 1–24.6%) than in 14 transient responders (mean: 28.2%, range: 7.4–75.5%) and in seven nonresponders (mean 41%, range: 8.8–86%) ($P = 0.036$). At the end of therapy the computed infected cell number was <100 cells/mL of extracellular fluid in all sustained responders (mean 18.4, range: 1.7–48), in three transient responders (mean: 3500, range: 1.52–17 500) and in none nonresponders (mean: 28 500, range: 1200–96 000) ($P = 0.003$). All three transient responders with <100 cells/mL received IFN alone, two developed a breakthrough of viral replication during treatment. Infected cells clearance was faster in patients with HCV genotypes 2 and 3 with an average half-life of 5.4 days (range: 3.8–14.3) vs. 8.7 days (range: 3.7–23.2) in genotype 1 ($P = 0.015$).
Conclusion: Our data suggest that, besides inhibition of viral replication, an efficacious clearance of the infected cells, as determined by fitting both HCV-RNA and ALT decline, is mandatory for sustained response. Accordingly, genotype 2 and 3 infected patients, who have a higher chance of response, show faster infected cell clearance. The analysis of infected cells dynamics by the new model might be useful to tailor duration of combination therapy in the single patient.

O359 Treatment of acute C hepatitis with pegylated interferon alpha-2b: preliminary results

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Objective: Infection caused by hepatitis C virus (HCV) leads to chronic infection in up to 80% of patients. Treatment with pegylated interferon significantly increased rates of cure compared with standard interferon. Acute C hepatitis may also benefit from interferon treatment. Identification of patients at risk of acute C hepatitis, such as intravenous drug users, sexual partners of HCV-infected patients and exposed health-care professionals may increase the detection rates of acute cases. We treated patients with acute C hepatitis with Peg-interferon a-2b for 12 weeks.
Methods: Inclusion criteria were documented seroconversion, positive HCV-RNA and elevated ALT levels with a known risk factor in the preceding 6 months. Patients were treated with Peg-interferon alpha-2b (1.5 μg/kg once weekly subcutaneously) for 12 weeks in an open, nonrandomised, prospective cohort study. ALT and HCV-RNA measurements were made at weeks 4, 12 and 24 weeks after the end of treatment. The primary endpoint was the sustained viral response (SVR).
Results: Twelve patients completed the treatment. Eight patients (75%) had HCV genotype 1. At baseline, median HCV-RNA level was 129 500 copies/mL (range: 3 000–3 100 000); eight patients were asymptomatic. Treatment was given within 30 days (range: 8–30) of the ALT level peak. At week 4 HCV-RNA was undetectable in all patients but one, and at week 12 all patients were HCV-RNA negative. Sustained viral response was achieved in eight of nine patients evaluable (88.8%). The only patient without SVR was the one positive for HCV-RNA at week 4.
Conclusions: Pegylated interferon alone administered for 12 weeks is effective in patients with acute C hepatitis. High rates of SVR were observed even in patients with genotype 1. Identification and treatment of acute C hepatitis may decrease the rate of chronic liver diseases. Further studies are needed to confirm the efficacy of a 12-week regimen with PegIFN in acute C infections.

O360 Mutation analysis of ISDR and V3 domains of hepatitis C virus NS5A region during interferon therapy with or without ribavirin

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Objectives: The hepatitis C virus (HCV) nonstructural 5A (NS5A) has been controversially implicated in the resistance of HCV to Interferon (IFN) therapy in clinical studies. In Japan, mutations in IFN sensitivity-determining region (ISDR) (aa 2209–2248) in the NS5A gene were associated with response to IFN therapy in patients infected with genotype 1b. In contrast, studies from Europe did not confirm such association. More recently, it has been suggested that the V3 domain (aa 2353–2379) outside the putative ISDR may also have amino acids changes that may be associated with response to IFN. In this study, the relationship between NS5A mutations in ISDR and V3 domains and virological response to therapy was investigated.
Methods: The NS5A gene was sequenced from 35 HCV genotype 1b infected patients, in a prospective clinical trial of IFN therapy and IFN plus Ribavirin combination therapy at D0, M3 and M6.
Results: In the ISDR domain, we did not observed significantly different variations in amino acids between responders

(1.52 ± 1.75, $n = 21$, range 0–6) and nonresponders (1.07 ± 0.83, $n = 14$, range 0–3), ($P = 0.778$), to therapy when tested before the beginning of treatment. In the V3 domain, we found more mutations in responders (6.48 ± 1.86, $n = 21$, range 2–11) than in nonresponders (4.71 ± 1.20, $n = 14$, range 3–8), before the beginning of treatment ($P = 0.001$). Few variations were observed during treatment in nonresponders (1.38 ± 1.51, $n = 8$, range 0–4) but the number of substitutions in responders to combination therapy was more important (6.60 ± 4.51, $n = 5$, range 1–13), after 3 months of treatment ($P = 0.019$).
Conclusion: Our results confirm that, in Europe, the sequence of the ISDR domain of HCV was not predictive for treatment success. However, we found that the V3 domain have greater variability in responders than nonresponders suggesting that this V3 domain needs further investigation.

O361 A nosocomial hepatitis B outbreak in Danish children

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Objectives: To investigate an outbreak of hepatitis B virus (HBV) infection in a paediatric haematology/oncology ward. The outbreak was disclosed subsequently to the incidental finding of HBV infection in a ward patient as the infecting viral strain (the epi-strain) was found to be identical to a virus isolated from the same ward 1 year earlier through a phylogenetic survey of consecutive HbsAg-positive samples in the country.
Methods: Patients admitted to the ward since the initial admission of the 'source patient' were screened for HBsAg and anti-HBc and HBV DNA was isolated and sequenced from infected individuals. Patients from whom the epi-strain was isolated were categorised as definite cases. Hygienic investigations were performed by the hospital infection control team.
Results: Of 175 patients admitted during the epidemic period, 155 were contacted and 133 were tested. Apart from the source, seven definite cases were identified. All case patients were immunodeficient, had a central venous catheter ($P = 0.0007$) and their stay at the ward clustered in time. Based on minute review of hospital records, transmission most likely took place on at least three occasions. In periods of high bed occupancy rates the ward medication room was used for blood sampling and intravenous medication. Following the re-establishment as a 'clean' room, including the replacement of multidose vials with single dose vials; no new cases were identified during 15 months of follow-up. HBV-infected patients remained asymptotic and HbeAg-positive during follow-up.
Conclusion: Case patients were probably infected through contaminated injections possibly via multidose vials accidentally contaminated in the medication preparation room. Molecular epidemiological surveillance of blood-borne viruses may identify unsuspected transmission routes and thereby reduce nosocomial transmission.

O362 Flares of hepatitis, circulating hepatitis B core (HBc)-specific T cells, and sustained virologic response to antiviral therapy in chronic hepatitis B patients

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Objectives: To test whether HBc-specific immune response plays a role in the sustained suppression of hepatitis B virus (HBV) after interruption of antiviral therapy.
Methods: Peripheral blood lymphocyte proliferation in response to autologous monocyte-derived dendritic cells pulsed with recombinant HBcAg was longitudinally assessed in 21 chronic hepatitis B patients during and after a 1-year course of pegylated interferon-α, alone or in combination with lamivudine. *In vitro* lymphocyte proliferation was measured by bromodeoxyuridine incorporation after 7-day culture and FACS analysis. Minimum 12 samples per patients were studied employing one single dendritic cell preparation. Eleven patients presenting HBV-DNA reduction greater than three logs at the end of a 6-month therapy-free follow-up (responders) were compared with the remaining patients who again attained pretherapy HBV-DNA levels (nonresponders).
Results: HBc-specific responses, both in CD4 and CD8 circulating T cells, were transiently detected during therapy and after therapy discontinuation in the majority of patients. Magnitude and frequency of responses did not significantly differ between responders and nonresponders. After therapy discontinuation, eight nonresponders presented vigorous HBc-specific T-cell responses. In five of them, significant elevations of transaminase levels occurred in temporal association with the presence of HBc-specific T cells in the circulation. In contrast, only one of the nine responders with detectable HBc-specific T cells in the circulation showed elevated transminase levels ($P < 0.05$ vs. nonresponders).
Conclusions: These data suggest that: (i) chronic hepatitis B patients are able to mobilize and expand HBc-specific T cells; (ii) the release of HBc-specific T cells is not invariably associated with flare-ups of hepatitis; (iii) the presence of HBc-specific T cells in the circulation in the absence of transaminase elevations is associated with prolonged suppression of viral replication.

O363 A new model of HBV infection dynamics shows a multiphasic decay of viral load and a short half-life of circulating virus in anti-HBe positive patients treated with lamivudine

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Objectives: Standard biphasic model of viral dynamic applies only partially to HBV patients. Our aim was to analyse the dynamics of HBV infection during Lamivudine (100 mg/die) in anti-Hbe-positive chronic hepatitis B using a new bio-mathematical model that can explain multiphasic decay patterns.
Methods: In 11 patients ALT and HBVDNA levels were measured (Amplicor HBV Monitor) at baseline, hourly in the first day, at 1, 2, 4 or 5, 7, 14, 21, 28, 45 and 60 days upon treatment and monthly thereafter. Experimental data were fit into a model in which: (i) the effect of the drug on virus production is described by a block followed by an exponential decline to an asymptotic value; (ii) the immune clearance of the infected cells is determined by the decline of alanine aminotranferase (ALT) and HBV-DNA and diminishes over time due to the negative feedback of the infected cells reduction; (iii) the number of infected cells at the beginning of therapy is estimated by the value of their immune clearance computed in the first month and that of ALT at baseline.
Results: Three phases of HBV-DNA decline were observed in eight of the nine analysable patients (two patients with minor fluctuation of ALT/HBV-DNA could not be analysed). During the 1st phase (days 0–2), mainly related to the block of virus production (median drug effectiveness value: 0.910, range: 0.320–0.999), the free virus decay constant was on average 2.38 days-1 (range: 1.70–2.91) with a half-life of circulating viruses of about 7 h (range: 5.7–9.8). In the 2nd phase (days 2–14) HBV-DNA decline was slower, with a median virus production decay constant of 0.34 days-1 (range: 0.00–0.75) and a median infected cells decay constant of 0.05 days-1 (range 0.028–0.12) corresponding to an infected cells half life of about 14 days (range: 6–25). A 3rd slowest phase of viraemia decline (from day 14 on) could be documented as long as HBV-DNA remained detectable (52 to >360 days) in seven patients. In this phase virus production per infected cell reaches its asymptotic value and viraemia decline depends only from the immune clearance of the infected cells.
Conclusions: HBV dynamics was successfully described in 80% of patients by this model. The circulating virus half life resulted to

be three to four times shorter with a narrow range of variability than reported previously. This finding could be due to the earlier and frequent sampling or, less likely, to peculiar features of the HBeAg defective HBV infection.

O364 Control of hepatitis A outbreaks before and after the era of routine childhood hepatitis A vaccination

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Objectives: Hepatitis A (HA) outbreaks occur in well-defined endemic communities. The World Health Organization recommends routine HA childhood vaccination in countries with intermediate endemicity and vaccinating young children in communities with recurrent outbreaks. Routine childhood HA vaccination was implemented in Israel in 1999. We observed two large HA outbreaks among young children in an endemic community before and after routine HA vaccination.
Patients/methods: A community-wide (118 cases) outbreak in 1996 was controlled through mass active vaccination of 1133 children born between 1991 and 1995. Subsequently, children who were born after 1 January 1998 were routinely vaccinated with two doses of Havrix® Junior vaccine at ages 18 and 24–30 months. After 31 months without HA cases, a second outbreak occurred in 2000.
Results: Altogether, 65 children were diagnosed clinically and confirmed serologically, nine (13.8%) were hospitalised and recovered. The specific attack rates were 13.6, 5.5, 1.65 and 0.97% for the 1996, 1997, 1998 and 1999 cohorts, respectively ($P < 0.01$). Most cases (90%) occurred in the unvaccinated 1996–1997 cohorts. Outbreak control measures included enhancing hygiene measures, catch-up immunisation and modification of the HA immunisation routine by giving the first vaccine dose at 12 months. Immunisation coverage for the first dose was 82 and 94% for 1996–1997 and 1998–1999 cohorts. No HA cases were observed since June 2000 and intensive follow-up continues.
Conclusions: (i) With implementation of routine childhood HA vaccination, it is essential to perform catch-up vaccination of young children in communities with recurrent outbreaks. (ii) Lowering the age of the first dose of vaccine to 1 year provides rapid protection, as no morbidity was reported in the young age group.

O365 20 years hepatitis A sero-epidemiology in Belgium

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Objectives: Five epidemiological patterns (very high, high, intermediate, low and very low) become apparent when incidence rates and age-specific antibodies to HAV (anti-HAV) prevalence are examined. This study evaluated, for a 20-year period, the age distribution of the prevalence of immunity to HAV, and the incidence of acute HAV infections in Belgian populations.
Methods: General population (GP) age-specific anti-HAV prevalence in 2000 were compared with the 1979, 1989 and 1994 prevalence.
Results: A clear epidemiological shift showing decreasing prevalence in the youngest age groups was found. In 1979 some 50% of the 30–34 years age group population were positive. In 1989 50% of immune individuals was reached in the age group 35–39 years and shifted in 1994 to the age group 40–44 years. In 2000 50% of immune individuals was only reached after the age of 45 years. So we can conclude that, over a period of 20 years, prevalence pattern shifted from intermediate to low.

For selected populations different, social status linked, epidemiological patterns were seen. In emigrants, mostly from Mediterranean origin, we still see an intermediate pattern. Health care workers, with an over-representation of higher social class, have a significantly lower prevalence than the GP. The incidence of clinical acute viral hepatitis A was lower in 2000–2001 than in the periods 1982–1984 and 1991–1992. The average age of the patients rose significantly compared with the two other periods. This proves that with the decreasing endemicity of HAV, the average age of exposure and infection has shifted to older age groups.
Conclusion: In Belgium the number of unprotected individuals younger than 50 years increased in the past 20 years. This number will still increase in the coming years. Considering the more severe course of the disease as age increases, vaccination may become an important strategy to protect susceptible individuals (i.e. paediatric nurses, catering personnel, travelers).

Epidemiology and management of community-acquired pneumonia

O366 Epidemiology of community-acquired pneumonia in Belgium

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Background: In the diagnosis of pneumonia the lack of a clear distinction between typical (*S. pneumoniae*) and atypical (*C. pneumoniae, M. pneumoniae, L. pneumophila* and viral agents) pneumonia is a permanent cause of difficult diagnostic and therapeutic decisions. There is a lack of information of the prevalence of atypicals in Belgium.
Methods: During the winter period 2000 and 2001 consecutive patients with an X-ray documented community-acquired pneumonia (CAP) were included in 10 centres (GPs and hospitals) participating in a multicentre study. Demographic and clinical data were collected. Conventional microbiological techniques and PCR were performed on respiratory samples for the detection of *M. pneumoniae* and *C. pneumoniae* (all except for three, where real-time was performed). Real-time PCR was performed for the detection of *S. pneumoniae, L. pneumophila*, Influenza A and B, RSV and para-influenza 1 and 3 viruses
Results: CAP was documented in 147 patients (44 out-patients and 103 patients needing hospitalisation). An aetiologic agent was detected by PCR in 67 patients (45.6%). Bacteria were found in 59 of 147 (40.1%) and viruses in 16 of 143 (11.2%) tested patients. Combined infections (bacterial–bacterial, bacterial–viral or viral–viral) were found in 13 cases (8.8%). Of the bacterial causes, *S. pneumoniae* was found in 46 (31.3%) and the atypical bacterial agents *M. pneumoniae, L. pneumophila* and *C. pneumoniae* in 17 (11.6%) cases (respectively 9.5, 2.0 and 0%). Of these 59 patients, 13 (22.0%) had taken antibiotics prior to consultation (average 8 days before) Of the viral causes, influenza A and B, RSV and para-influenza 1 and 3 were respectively found in 3.5, 0.7, 4.2, 1.4 and 2.8%. Of those 16 patients six (37.5%) had previously received antibiotics (average: 10 days before).
Conclusion: Atypicals were seldomly found in CAP patients. Rapid real-time detection methods are helpful in the detection of a bacteriological agent (particularly in patients with prior antibiotic therapy), and in installing appropriate antibiotic treatment.

O367 Value of real-time PCR for the aetiologic diagnosis in adults hospitalised with lower respiratory tract infections

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Objectives: Nucleic acid amplification techniques are increasingly implemented in diagnostic settings. However, few hospitals have

introduced these techniques in the routine setting of diagnosis and treatment of lower respiratory tract infections in adults.
Methods: To determine the diagnostic value of real-time PCR techniques in a routine setting, we collected nose-throat samples of immunocompetent patients admitted in our hospital for antibiotic treatment of lower respiratory tract infections. The samples were evaluated by virus culture and by real-time PCRs for adenoviruses, coronaviruses, enteroviruses, influenzavirus A/B, parainfluenzavirus 1–4, rhinoviruses, RS virus and *Chlamydia pneumoniae, Legionella pneumophila* and *Mycoplasma pneumoniae*. In addition, blood and sputum samples were taken for culture and acute and reconvalescent serology samples were evaluated for respiratory viruses (influenza virus A/B, para-influenza virus 1–4, RS-virus, adenovirus) and atypical pathogens (*M. pneumoniae, C. pneumoniae, L. pneumophila*).
Results: 63 consecutive patients (34 men, age 63 ± 16) were included of whom 29 (34%) had pneumonia (chest X-ray showing infiltrate). Respiratory viruses were detected by culture in eight patients, by real-time PCR in 19 patients. (30%). The most frequently detected agents were influenza A ($n = 9$) and coronavirus ($n = 5$). Viruses were more frequently detected in patients without pneumonia (15/34; 44%) than in patients with pneumonia (five of 29; 17%) ($P = 0.02$) In one patient influenza A virus was cultured, but not detected by real-time PCR. In nine of 20 patients (45%) with a virusinfection as determined by real time PCR, respiratory complaints were present for 3 days or less. Added to sputum and blood cultures, real-time PCR increased the number of patients in which a causative micro-organism was identified from 14 (22%) to 27 (43%).
Conclusion: Viral agents play a substantial role in the aetiology of hospitalised lower respiratory tract infections. Real-time PCR increases the number of aetiologic diagnoses in immunocompetent adults admitted with lower respiratory tract infections with 21%.

O368 Significance of bronchoscopy and invasive techniques in the diagnosis of community-acquired pneumonia

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Objective: The role of fibreoptic bronchoscopy in the diagnosis of CAP is not well defined.The aim of this study was to evaluate the diagnostic yield of protected specimen brush (PSB) and bronchoalveolar lavage (BAL) cultures in hospitalised patients with CAP and to compare the results with sputum cultures.
Methods: Ninety-three patients with a presumpive diagnosis of CAP were enrolled. CAP was defined as symptoms of lower respiratory tract infection along with new infiltrate on chest x-ray in a patient not hospitalised for the last 2 weeks. All patients underwent fibreoptic bronchoscopy (FB) with PSB and BAL before antibiotic treatment. Gram-staining, quantitative cultures of sputum, PSB and BAL and identification of microorganisms were performed according to NCCSL. McNemar's test was used for statistical analysis.
Results: Of 93 patients, 33.3% had a positive sputum culture, 62.3% had a positive PSB culture and 72% had a positive BAL culture. *S. pneumoniae* was the most frequent pathogen (18.2%). *H. influenzae* developed in 8.6% of patients, *K. pneumoniae* and *P. aeruginosa* in 3.22% each, *L. pneumoniae, M. catarrhalis, H. parainfluenzae, S. aureus* and *S. mitis* in 2.15% each. *M. pneumoniae* was isolated in PSB and BAL in 2.15% of patients through antigenic identification techniques. Mixed infections were detected in 11.8% of patients and 6.4% of patients developed *M. tuberculosis*. Nontuberculous mycobacteria were isolated in three patients and *N. asteroides* in one patient. During the work-up an alternative diagnosis emerged for five patients. Mortality was 5.37%.
Conclusions: (i) A statistically significant difference was found concerning the diagnostic yield of PSB and BAL culture compared with sputum culture. ($P < 0.001$) (ii) PSB and BAL contributed to the early diagnosis of tuberculosis in nonhighly suspected patients. (iii). Treatment modification supervened in 33% of patients due to bronchoscopic results.

O369 Diversity of *Legionella* subtypes in cooling towers. Why only one environmental strain causes the clinical cases

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Objectives: Outbreaks of Legionnaires' disease (LD) are frequently associated with cooling towers. Although different strains may coinhabit a cooling tower, only one strain may be responsible for an outbreak. In 2002 an outbreak of community-acquired LD was reported in Mataró (Spain) involving 113 patients. All the clinical isolates ($n = 10$) exhibited the same chromosomal DNA subtype. However, two chromosomal DNA subtypes were found in the cooling tower responsible for the outbreak, one of which matched the clinical isolates. In order to know why one only environmental strain caused clinical cases, we decided to study the growth of both environmental strains in BCYE broth and a cell culture.
Methods: The same quantity of the two strains of *Legionella* were cultured in BCYE broth. The broth was maintained at 37°C with agitation for 70 h. An aliquot of the coculture was periodically plated in GVPC agar. Ten colonies were isolated from each. The chromosomal DNA subtype of these colonies was determined by pulsed-field gel electrophoresis (PFGE). Macrophages were also infected with an identical amount of the two strains of *Legionella*. At 24, 48 and 72 h the culture was lysed and plated in GVPC the bacterial suspension. Ten colonies of each well were analysed by PFGE.
Results: During the first hours of growth the proportion of the two strains was similar in either the microbiological culture and the cellular experiment. However, over time the proportion of the strain found to cause the outbreak increased in comparison with the other. At the end of the experiment almost all the strains isolated presented the chromosomal DNA subtype corresponding to the strain responsible for the outbreak. Comparing these data in the growth curve we observed that the outbreak causing strain clearly predominated in the postexponential phase of growth .
Conclusions: The environmental isolate causing most of the clinical cases in the Mataró outbreak was able to grow and survived better in two different experimental models compared with another isolate found in the same cooling tower system. This better adaptation to the environmental conditions suggests greater virulence which may explain both the experimental and clinical data observed.

O370 Current clinical outcomes in hospitalised patients with community-acquired *Legionella* pneumonia

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Objectives: It has been suggested that urine antigen testing and fluoroquinolone treatment might have improved outcomes in patients with community-acquired *Legionella* pneumonia (LP). The aim of this study was to analyse current clinical outcomes in patients with LP; including response to antibiotic therapy, development of in-hospital complications, and mortality.
Methods: Prospective observational study of consecutive nonseverely immunosuppressed adults hospitalised with community-acquired pneumonia (CAP) from February 1995 to June 2003 in a university hospital.
Results: Of 1869 patients hospitalized with CAP, 125 (6.7%) had LP (urine antigen test 107, fourfold rise in serum antibodies 78, and positive culture 40). There were 109 males (87.2%); mean age of 56 years (SD 20.9). Sixty-two patients (50%) had comorbid conditions, 57 (46%) presented with multilobar pneumonia, and 59 patients (47%) were classified in risk classes IV and V, defined by the Pneumonia Severity Index. A total of 110 patients (88%) received an appropriate empirical therapy, including a macrolide (erythromycin or clarithromycin) in 80 (73%) cases, levofloxacin in 29 (26%) cases, and both in one case. Patients receiving levofloxacin had a faster time to defervescence than patients receiving macrolides (2.5 days vs. 5.8 days, $P < 0.001$) and to clinical stability according to predefined criteria (3.7 days vs. 6.6 days, $P = 0.006$),

but no differences were found regarding the development of complications (31% vs. 31%, NS) and mortality (<30 days) (3.4% vs. 5.0%, NS). Length of hospital stay was 9.6 days in patients treated with levofloxacin and 14.4 days in patients receiving macrolides ($P = 0.010$). Overall, 39 of 125 patients (31%) with LP had one or more in-hospital complications; mainly worsening of comorbid conditions (26 cases), ICU admission (19), respiratory failure requiring mechanical ventilation (11), renal failure (8), and pleural empyema (2). Early mortality (<48 h) occurred in four patients (3.2%) and overall mortality (<30 days) in seven patients (5.6%).
Conclusion: Our data suggest that, in hospitalised patients with LP, levofloxacin produces a faster clinical response than macrolides. LP is still associated with significant morbidity, but current mortality is substantially lower than that traditionally reported.

O371 Outbreak of pneumococcal pneumonia in a retirement home in France in 2003

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Streptococcus pneumonia is the main causative agent of bacterial pneumonia. However, outbreaks of pneumoccocal disease are rarely described in France. We describe an outbreak of invasive pneumococcal pneumonia occurring in a retirement home with 94 residents and 63 staff. In France, national recommendations for adult pneumococcal vaccination are restricted to specific medical conditions.
Methods: The health authorities in Strasbourg were alerted when four cases of invasive pneumococcal infection were detected, over the space of a few days, in a retirement home in the town. A retrospective cohort study was carried out among the residents of the retirement home. All residents were examined for respiratory symptoms and fever. Confirmed cases had infectious signs and isolation of *S. pneumonia* in blood or CSF or presence of urinary streptococcus antigens. Probable cases had only clinical and radiological signs of pneumonia. We collected information on clinical signs, co-morbidities, living habits, self-sufficiency, and social activities. A nested case-control study was carried out to identify risk factors for pneumococcal infection. Previous medical history, contact with ill residents or visitors and previous vaccination for pneumococcal diseases were investigated. Active case finding among staff also took place. Oropharyngeal samples from all staff and residents in the week following the epidemic were tested for the presence of *S. pneumoniae*.
Results: Between 13 and 17 October 2003, we observed nine confirmed and two probable cases of pneumococcal invasive infection among the residents (attack rate = 11.7%). No cases were observed among the staff. Carriage of *S. pneumonia* was found only in one nonsymptomatic resident. *S. pneumoniae* serotype 4 was isolated in five cases. One case had meningitis. All other cases had pneumonia and/or bacteraemia. Three of the 11 cases died. All cases were women with a mean age of 89.3 years. None of the cases had received the pneumococcal vaccine in the last 5 years while four of them were eligible for pneumococcal vaccination according to the national recommendations. The cases were distributed over all five floors of the building. No common activity was identified for all cases. Data from cohort and case-control studies will be presented.
Conclusion: The low level of vaccination among the cases suggests that the applicability and pertinence of current vaccination recommendations need to be examined.

O372 Early administration of antibiotics in patients with community-acquired pneumonia in the emergency room

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Objective: To evaluate the impact of initial choice of antibiotics and the time door-antibiotics to the clinical outcome for the patients with community acquired pneumonia (CAP) at the Emergency Departments (ED).
Methods: (i) Design – multicentre prospective study. (ii) Setting – 14 ED of Catalunya (Spain) and Principality of Andorra. (iii) Period: from 20th January to 1st July 2003. Patients: All patients >18 years admitted through the ED with a diagnosis of CAP. The diagnosis of CAP required the following: (i) new infiltrate on chest radiograph; (ii) clinical evidence of pneumonia, with presence of at least two of the following – temperature $\geq 38^{\circ}C$, cough, purulent sputum, pleuritic chest pain, dyspnoea, confusion. Patients were excluded if they had an immunosuppressive illness, hospitalisation in the last 14 days or another alternative diagnosis. Data was collected for demographic information, triage time, previous or current antibiotic administration, time to antibiotic administration, intial antibiotic regime and clinical outcome at 30 days. The study was approved by the Sant Pau Ethic's Comittée.
Results: A total of 1034 patients were entered into the study. One hundred had an immunosupressive illness or an alternative diagnosis and were excluded from the analysis. Two hundred patients received prior ambulatory antimicrobial treatment. This included: aminopenicillins (83), macrolides (40), quinolones (31) and oral cephalosporins (15). Initial empiric antimicrobial treatment after admission to the ED comprised the following: levofloxacin (343), amoxicillin–clavulanate(244), third generation cephalosporin + macrolides(153) and others. The mean time to administration of first dose of antibiotics was 4 h 39 min (SD 3 h). Clinical outcome at 30 days was: complete recovery in 630 (77%), partial recovery in 113 (14%), treatment failure 20 (3%) and 54 (7%) deaths. There were significant differences between the mean time to administration of first dose of antibiotics and mortality (<4 h: 45%, 4–8 h: 22%, >8 h: 5%, unknown: 30%), and mortality was statistically different among the various antibiotics regimes (cephalosporins 9%, cephalosporins + macrolide 7%, aminopenicilins 5%, levofloxacin 4%).
Conclusions: (i) The initial choice of antibiotics may influence the clinical outcome. (ii) The administration of antibiotics within 4 h of admission in the ED, as an isolated factor, does not reduce the mortality in adult patients with CAP. (iii) Future studies using a multivariate analyses may be undertaken.

O373 Treatment with sequential intravenous/oral moxifloxacin was associated with faster clinical improvement and earlier discharge from hospital in CAP patients requiring initial parenteral therapy compared with standard therapy

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Objectives: To compare the efficacy and safety of sequential intravenous (IV)/oral (PO) moxifloxacin (MOX) and high-dose ceftriaxone with or without erythromycin (CEF) in patients with community-acquired pneumonia (CAP) requiring parenteral therapy.
Methods: This controlled, multicentre, randomized, prospective, nonblinded phase-IIIb study was performed in 54 centres in Germany, France, Greece, Lithuania and Poland. Adult patients with signs and symptoms consistent with bacterial CAP requiring initial parenteral treatment were randomised to MOX (400 mg once daily) IV, possibly followed by oral tablets or to ceftriaxone (2 g IV once daily) with or without erythromycin (1 g IV q6 h or q8 h), for 7–14 days. The primary efficacy variable was the clinical response at the test-of-cure visit (TOC) 5–20 days after end of study therapy.
Results: Of 397 randomised patients 317 (79.8%) were evaluable for efficacy. Mean age was 58.1 years (range 18–96). 59 of 156 (37.8%) patients in the CEF group had additionally received erythromycin. 81% of MOX-treated patients were switched to oral drug at a mean of 5.7 days. Clinical cure at TOC was achieved in 138 of 161 patients (85.7%) in the MOX group and in 135 of 156 patients (86.5%) in the CEF group (95% CI, −7.9 to 7.1%). After 3–5 days of therapy only the MOX-treated group had achieved an at least 50% decrease in the mean serum concentration of C-reactive protein from baseline (CEF group 42%). In patients with fever (>38.5°C) at entry, defervescence occurred significantly earlier in the MOX group than in the CEF group at a mean of

3.8 days vs. 4.8 days ($P = 0.0027$). Furthermore, a faster improvement of symptoms in the MOX group was recorded by the patients in standardised diaries. Due to IV-to-oral switch, overall IV treatment duration was shorter for MOX than for CEF (5.4 days vs. 9.5 days). Mean length of stay in hospital (LOS) was significantly shorter in the MOX group than in the CEF group (9.8 days vs. 11.1 days; $P = 0.0005$).
Conclusions: In adult patients hospitalized with CAP, sequential IV/PO MOX was clinically equivalent to high-dose ceftriaxone with or without erythromycin. However, faster defervescence and faster symptoms' improvement was observed in MOX treated patients. Switch to oral treatment was feasible in most patients without compromising clinical efficacy. The faster clinical improvement observed in MOX-treated patients allowed an earlier discharge from hospital with potential cost savings.

O374 Management of community-acquired lower respiratory tract infections in the emergency department. A prospective 2-year observational study in over 170 centres throughout France

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Prevalence of community-acquired lower respiratory tract infections (LRTI) is high in emergency departments (ED) worldwide. Purposes of this survey of 11 days in March 2002 (I) and 8 days in March 2003 (II) are (i) describe the epidemiology of LRTI seen in the ED (ii) identify management of patients with acute exacerbation of COPD and pneumonia (CAP) and (iii) determine their short-term outcome in II.
Methods: Consecutive LRTI patients aged 18 years or more were included. Anthonisen criteria, Fine score and their usefulness for management were circulated during I and II and antibiotics guidelines for LRTI issued by Health Care Authorities were handed during II only.
Results: A total of 137 EDs in I and 114 in II (74 participated in I and II) included 1603 and 1552 LRTI patients. aged 62.8 ± 19.0 and 61.9 ± 21.1 years, sex ratio 58% M in both I and II. Among them, 844 and 689 had CAP, 554 and 325 COPD, 205 and 538 miscellaneous. Based on a total of 126 000 visits in I and 98 000 in II, LRTI accounted for respectively 2.9–3.4% of nontrauma patients. With a ratio of 2 CAP/1 COPD in both instances. Severity of disease was based on Fine for CAP (Fine 1, 18 and 19%; 2, 19 and 16%; 3, 24 and 22%; 4, 27 and 31%; 5, 10 and 13%) and for COPD, on the presence of chronic respiratory insufficiency (COPD+ 40.8% vs. 55%) or not. COPD are older than CAP patients. (72.1 ± 14.0 years vs. 66.6 ± 20.3 years in I and 71.0 ± 13.8 years vs. 63.5 ± 21.0 years. in II) and more often hospitalised (90% vs. 79.9% in I and 78% vs. 76% in II). However, more CAP than COPD received antimicrobials (AM) in the ED (83% vs. 58% and 85% vs. 74%; $P < 0.001$) and AM combination was more frequent in CAP than COPD (25% vs. 20% and 25% vs. 24%). There is a significant trend to discharge patients between I and II for CAP (20% vs. 24%) and COPD (10% vs. 22%; $P < 0.001$). At day 5 of hospital admission during II (523/689 CAP and 253/325 COPD), patients were discharged in 14 and 16%, still hospitalised in 75 and 72% and dead in 4 and 3% of cases. AM prescribed in the ED were confirmed for 72% of patients hospitalised.
Conclusions: (i) These two surveys confirm that French EDs are the first management site for many LRTI patients; (ii) The trend to discharge patients of mild to moderate severity has increased between 2002 and 2003. Management guidelines specifically designed for the ED, with emphasis on orally administered AM and safety, would probably enhance ambulatory treatment of LRTI patients.

O375 Recently formulated guidelines for empiric therapy of community-acquired pneumonia increase antibiotic use with 63%

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Objectives: New BTS and IDSA guidelines advocate empirical treatment with both β-lactams and macrolides for patients admitted to hospital because of community-acquired pneumonia (CAP). We evaluated the impact on antibiotic usage of such a strategy when introduced in the Netherlands.
Methods: Patients admitted with severe CAP (signs and symptoms of CAP and chest X-ray showing infiltrate) but not needing mechanical ventilation to six hospitals in the Netherlands were prospectively studied for 28 days. Sputum and blood samples were cultured, acute and reconvalescent serology samples were evaluated for atypical pathogens (*Mycoplasma pneumoniae, Chlamydia pneumoniae* and *Legionella pneumophila*) and urine samples were tested for *Legionella* and pneumococcal antigen. Demographic, clinical and treatment data were documented. Subsequently, we evaluated costs and adequacy of treatment assigned, should BTS-guidelines have been followed. We defined antibiotic usage as the number of defined daily doses (DDD) of a standard 10 day antibiotic course.
Results: A total of 232 consecutive patients (mean Fine 114 ± 28; age 69 ± 15) were included between July 2000 and May 2003. Empirical therapy was β-lactam monotherapy in 82.2%, β-lactam in combination with macrolides, quinolones or aminoglycosides in 9% and quinolone monotherapy in 0.5%. Overall mortality was 8.2%. Antibiotic usage was 2601 DDD. 26 patients (11.2%) had atypical infection and empirical antibiotic treatment did not cover atypical infection in 17. Five of these showed a delayed clinical response, but none (0/5) died or needed mechanical ventilation. Should BTS guidelines have been followed, β-lactam/macrolide combination treatment would have been given to 83%, resulting in antibiotic usage of 4200 DDD, an increase of 63%. In three patients with atypical infection, initial antibiotic treatment would not have covered atypical infection.
Conclusion: While this population had a high mean Fine score, overall mortality remained low with current empiric therapy recommendations. The clinical benefit of β-lactam/macrolide empirical treatment for CAP in this population remains to be determined, but such a strategy certainly increases antibiotic usage, possibly enhancing development of antibiotic resistance.

Biofilms

S384 Biofilm biology – role of quorum sensing

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The Gram-negative bacteria *Pseudomonas aeruginosa* and *Burkholderia cepacia* are opportunistic human pathogens that are responsible for severe nosocomial infections in immunocompromised patients and are the major pathogen in cystic fibrosis. Both bacteria utilize quorum-sensing (QS) systems, which rely on N-acyl-homoserine lactone (AHL) signal molecules, to control the expression of virulence factors and to regulate biofilm development. It is shown that the two bacteria are capable of

unidirectional communication by the aid of AHL molecules and that this interaction appear to be of profound importance for the virulence of the mixed consortium. Efficient treatment of *P. aeruginosa* or *B. cepacia* infections is often hampered by the intrinsic resistance of the organism against a large range of antibiotics. The design of novel agents that specifically target the quorum-sensing systems of the two organisms is reported. In the presence of respective compounds the two opportunistic pathogens were greatly attenuated in different animal pathogenesis models.

Current problems in clinical mycology (Symposium arranged with EFISG)

S388 Mucormycosis: pathogenesis, diagnosis, management

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The Zygomycetes constitute a class of organisms that are characterised by the presence of sparsely septated, broad and polymorphic hyphae in tissue. They are divided into two orders, the Mucorales and the Entomophthorales. The majority of cases of zygomycosis are caused by the Mucorales. While *Rhizopus* spp. are the most commonly implicated organisms, an expanding spectrum of other Zygomycetes has been reported during the past decade, including but not limited to *Mucor, Rhizomucor, Absidia, Apophysomyces, Cunninghamella* and *Cokeromyces*. In immunocompromised or debilitated hosts, the Mucorales have a high propensity to invade blood vessels, resulting in a rapidly deteriorating clinical course refractory to antifungal therapy with extremely high mortality. Infection may develop at various tissue sites and may be classified as rhinocerebral; pulmonary; cutaneous; abdominal-pelvic and gastric; and disseminated disease. Rhinocerebral, pulmonary and disseminated zygomycosis are the most frequently encountered entities. The clinical and radiographic presentation resembles that of invasive infections by other filamentous fungi, in particular, *Aspergillus* spp. Therefore, the diagnosis of zygomycosis entirely relies on conventional microbiological, microscopic and histological demonstration of the organism in specimens from clinically suspected sites, with molecular detection methods being developed at a rapid pace. Always, a detailed radiographic evaluation is needed to assess the anatomic extent of invasive zygomycosis and to guide surgical resection of infected tissue. The mainstay of therapy of invasive zygomycosis consists of aggressive surgery of amenable lesions and high dosages of amphotericin B (1–1.5 mg/kg/day). A valid option is to use high dosages (5 mg/kg/day and greater) of an amphotericin B lipid formulation upfront to deliver the largest amount of drug to the site of infection and to preserve renal function. While echinocandins are inactive as single agents, antifungal triazoles have organism-dependent activity that is worthwhile to be explored further. Early clinical data suggest that the investigational triazole posaconazole may be useful for treating invasive zygomycosis. Critical to successful outcome of zygomycosis is the reversal of immunological or metabolic defects that precipitated its development.

S389 Fungal bone infections

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Fungal bone infections are rare. Worldwide ditributed infections such as those caused by *Candida* sp., *Aspergillus* sp. and *Cryptococcus neoformans* should be separated from those which are endemic (*Histoplasma* sp., *Blastomyces dermatitidis, Coccidioides immitis*, sporotrichosis and fungal mycetoma or dematiaceous fungi). Bone involvement can result from haematogenous dissemination or be secondary to skin infection followed by a contiguous extension. Evolution of the disease is most often subacute or chronic and radiological images are nonspecific. Diagnosis of fungal bone infection should be emphasized in some high-risk patients such as those who are immunocompromised or those who have travelled and stayed in endemic areas in the presence of subacute bone infection nonresponding to antibiotics. The diagnosis is obtained through direct examination or culture of a bone sample in association at least for some fungal diseases by the serum detection of fungal antigen or antibody. At the acute stage, the treatment is often based on the combination of surgery and antifungals, most often including amphotericin B or its lipid derivatives. New antifungals and particularly voriconazole recently gave very encouraging results in bone aspergillosis in our hands. In addition, caspofungin which has been shown to be active against biofilm producing *Candida* sp. strains could be promising for the treatment of fungal infected articular prosthetic devices. Systemic azoles are the long-term therapy of choice of such very difficult to treat infections.

Antimicrobial susceptibility testing of difficult organisms

S394 Mycoplasmas and chlamydia: requirements for antibiotic susceptibility testing and results

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Mycoplasmas and chlamydia are fastidious microorganisms involved in urogenital and respiratory tract infections. They differ by their growth requirements, acellular specific media for mycoplasmas, cell culture methods for chlamydia. Because of their intrinsic resistance, only tetracyclines, macrolides and related antibiotics, and fluoroquinolones, are useful therapeutic agents. Acquired resistance to these drugs have been reported in clinical isolates of *Mycoplasma hominis* and *Ureaplasma* spp., two genital mycoplasmas. They concern mainly tetracyclines and at a lower extent, macrolides and related antibiotics and fluoroquinolones. Because of these resistances, antibiotic susceptibility testing is useful, mainly when mycoplasmas are isolated from immunosuppressed patients. *In vitro*, strains of *M. pneumoniae* resistant to macrolides or to fluoroquinolones have been selected. However, *in vivo*, only resistance to macrolides has been reported in a very small number of isolates. So, susceptibility testing of *M. pneumoniae* is not currently indicated except when new drugs are proposed. Several techniques can be used for mycoplasmas, broth or agar dilution, kits adapted to genital mycoplasmas, or eventually E-test for *M. hominis*. Few reports have described antimicrobial resistance in chlamydia. Rare cases of therapeutic failures in *Chlamydia trachomatis* infections have been attributed to multidrug heterotypic resistance, concerning only a small number of organisms. Homotypic resistance has not been detected in any

human *Chlamydia* species. *In vitro*, strains resistant to fluoroquinolones were obtained by serial passages of *C. trachomatis*, in the presence of ofloxacin and sparfloxacin. Furthermore, naturally-occuring antibiotic resistance has not been reported in *C. pneumoniae*. Considering these results, *Chlamydia* susceptibility testing, useful for new drugs, could be also interesting to explain some therapeutic failures in *C. trachomatis* infections. Conditions for susceptibility testing for *Chlamydia* must take into account the cell-line utilized, the time between infection and addition of antimicrobial, the inoculum used, the effects of multiple passage and the technology used to investigate antibiotic activity (immunofluorescence staining, flow cytometry, molecular-based test). Finally, whatever the organisms, mycoplasmas or chlamydia, the major drawback in evaluating the antibiotic susceptibility is the lack of standardised methodology.

Tick-borne diseases

S396 Epidemiology and ecology of tick-borne encephalitis

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Tick-borne encephalitis is caused by tick-borne encephalitis virus (TBEV; Flaviviridae, genus *Flavivirus*) which is distributed in an endemic pattern over a wide territory of Europe and northern Asia within the range of the main ixodid tick vectors, *Ixodes ricinus* (European subtype) and *I. persulcatus* (Siberian and far eastern subtypes). TBEV, as an arbovirus, relies on two different types of hosts for its transmission cycles: ticks which act as both virus vectors and long-term reservoir hosts, and vertebrates that amplify the virus infection by acting as a source of virus for feeding ticks. Reciprocal specific interactions between TBEV and tick vector, TBEV and vertebrate host, and, between tick vector and vertebrate host in the appropriate environment of the specific geographical area create in a concerted manner unique conditions for virus to perpetuate its transmission cycles and survive. TBEV survival is based on the intimate ecological association with *I. ricinus*, or alternatively, *I. persulcatus* ticks having three developmental stages (larvae, nymphs and adults) overlaping in their activity and feeding strategy with a preference for certain selected vertebrate hosts. The role of vertebrate species as amplifying hosts is highly specific with only a few species efficiently supporting TBEV transmission. Among tested species *Apodemus flavicollis* field mice were the most efficient amplifying hosts of TBEV. Coincident aggregated distribution of *I. ricinus* larvae and nymphs was highest on *A. flavicollis* as observed in western Slovakia. This specific feature consistently increased the number of infectible larvae feeding alongside potentially infected nymphs and was characteristic for tested natural foci of infection. Human infections occur via an infected tick bite, or, alternatively and less frequently, via drinking of row goat or sheep milk. Exposed groups such as forest workers are in TBEV endemic areas at high risk of infection and should be preferentially vaccinated.

S397 Clinical manifestations and sequelae of tick-borne encephalitis

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Tick-borne encephalitis (TBE) is the most important arbovirus in Europe with approximately 3000 reported cases annually, the Baltic States included. The acute infections can present as a subclinical infection or an unspecific fever. These patients are never diagnosed as TBE in clinical practise. But as much as one fourth of the infected will develop a second stage of disease with central nervous system (CNS) involvement. The clinical spectrum includes meningitis or meningoencephalitis with or without myelitic involvement. The lethality in Europe (western TBE-virus) are 0.5%. The patients have a protracted acute and convalescent phase compared with other viral aetiologies (e.g. enterovirus). The risk for permanent sequelae are high, up to 40%. The most common reported complaints included in this postencephalitic syndrom are different cognitive and neuropsychiatric symptoms, headache, dysphasia, ataxia and hearing disturbances. Approximately 5% of the patients develop permanent pareses (shoulder girdle, hemi- or tetraparesis). The acute clinical manifestations and the described postencephalitic syndrome will be reviewed in this lecture.

S398 Current and future possibilities for prevention of tick-borne encephalitis

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Tick-borne encephalitis (TBE) virus transmitted by ticks remains a serious health problem in Central and Eastern Europe. TBE virus is a member of the flavivirus family. The biology of the main vector ticks, *I. ricinus* and *I. persulcatus*, is explained as far as it is medically relevant. Since no specific therapy of TBE exists, vaccination against the TBE virus has been established for many years in TBE virus endemic countries. Two commercially available vaccines are used in Europe: new versions of Encepur, Chiron Behring, Germany; and FSME-IMMUN, Baxter, Austria. The conventional vaccination schedule consists of three doses at day 0, month 1–3 and 9–12 after the second dose. In 1971, the development of an inactivated vaccine was initiated by Prof. Ch. Kunz. Various versions of this first TBE vaccine (FSME-IMMUN) have been approved since 1976. In 1999 the preservative thiomersal was removed. In the year 2000 a new TBE vaccine (TicoVac) was introduced which was free of thiomersal and human serum albumin. This led to an increased reporting rate of high fever and cases of febrile convulsions in infants and small children. Human albumin was subsequently reintroduced into the vaccine formulation in 2001 and there was a dramatic reduction of adverse event reports with this amended formulation (FSME-IMMUN new) compared with TicoVac. A paediatric formulation is presented at half the antigen dose in a 0.25 mL volume (FSME-IMMUN Junior). The first licensed TBE vaccine specifically for children (Encepur K), was licensed in 1994 in Germany with a reduced antigen content from the adult preparation. Although very rare, a number of children had symptoms of allergic reactions, probably to the polygeline content. Therefore, an improved TBE vaccine was developed: Encepur Adults (0.5 mL), licensed for use in persons older than 12 years, containing 1.5 μg inactivated TBEV antigen (strain K 23) with 1 mg aluminium hydroxide. Encepur children (0.25 mL) contains 0.75 μg inactivated TBEV antigen with 0.5 mg aluminium hydroxide. These vaccines are also licensed for rapid immunization to be applied at days 0, 7 and 21. The cloning of the TBE virus genome has provided the basis for detailed studies on the molecular basis of virulence and evaluation of new vaccine concepts. This includes vaccines consisting of recombinant subviral particles, DNA vaccines and conventional attenuated vaccines.

S399 Containment of TBE: the Austrian experience

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In the prevaccination era, Austria had the highest recorded morbidity of tick-borne encephalitis (TBE) in Europe. This prompted us in 1971 to develop an inactivated vaccine in a co-operative research project with J. Keppie from the Microbiological

Research Establishment, Porton Down, England. Immuno (now Baxter) fortunately decided to take over the industrial production of the candidate vaccine (FSME Immun), which became commercially available in Austria in the spring of 1976. More than 25 years of experience with vaccination in Austria, where more than 38 million doses of the Austrian vaccine have been used, has demonstrated, that FSME Immun is both well tolerated and highly immunogenic. The rate of protection under field conditions after completing the whole series of three vaccinations exceeds 98%. Initially our attempts were mainly directed towards achieving a high vaccination coverage in the high risk population. However, by 1979, a year with a record incidence of cases, it became apparent that, as in most other countries, TBE had become a disease that is predominantly acquired during leisure activities. Subsequently a mass vaccination was started in 1981 which since then each year lasts for 5–6 months in the first part of the year. Due to this measure, the vaccination coverage of the Austrian population of 8 million increased from 6% in 1980 to about 87% in 2002. After the onset of the vaccination campaign, beginning in 1984 (vaccination coverage of the Austrian population about 40%), a more or less steady decline of the disease could be observed, from 677 cases in 1979 to an average of 59 annual cases in the years 1999–2003. In particular, vaccination programmes for children at mandatory school age in endemic areas had a substantial impact on the incidence of TBE. Whereas in 1971–1981 almost 19% of the patients were 7–14 years of age, in the years 1990–2000 the percentage in this age group had fallen to 2.3%. It is noteworthy that in some European countries an increase of TBE has been observed in recent years. For example, in our northern neighbour, the Czech Republic, a marked increase of disease has been recorded in the early nineties. In that country, even in high risk areas such as southern Bohemia, only about 10% of the population has been vaccinated. This level of vaccination coverage will not suffice to substantially lower the incidence of TBE as the Austrian experience has shown.

In vitro studies of antimicrobial agents, fitness and virulence factors of bacteria

O400 Sensititre YeastOne™ Colorimetric Antifungal Plate for testing voriconazole against isolates of *Candida* species: a comparison with the NCCLS M27-A2 microdilution reference method

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Objectives: The purpose of this study was to extend the evaluation of the commercial Sensititre YeastOne Colorimetric Antifungal Plate for susceptibility testing of *Candida* spp. to the new triazole voriconazole. The available National Committee for Clinical Laboratory Standards (NCCLS) microdilution method for the antifungal susceptibility testing of *Candida* spp. and *Cryptococcus neoformans* (M27-A2 document) may not be the most efficient and convenient procedure for use in the clinical laboratory. It has been demonstrated that the Sensititre YeastOne plate provides comparable MICs to those obtained by the NCCLS method for testing yeasts and has been recently cleared by the FDA for testing fluconazole.
Methods: We compared MIC values obtained simultaneously by Sensititre YeastOne Colorimetric Antifungal Panel and NCCLS M27-2A broth microdilution methods after 24- and 48-h of incubation for voriconazole and reference agent, fluconazole. The 102 selected clinical isolates evaluated included 25 *Candida albicans*, six *C. glabrata*, 22 *C. krusei*, 21 *C. lusitaniae*, 19 *C. parapsilosis*, five *C. tropicalis* and other four less common *Candida* spp. Colorimetric MICs of both antifugal agents were the first blue (no growth) or purple well.
Results: Two comparisons of MIC pairs were evaluated to obtain percentages of agreement (±2 dilution range): 24-h colorimetric vs. 24- and 48-h NCCLS MICs. The agreement between the methods for voriconazole MICs was 97% when colorimetric MICs were compared with 24-h NCCLS MICs; the agreement was lower (90%) when 24-h colorimetric values were compared with 48-h NCCLS MICs. The agreement for fluconazole was excellent with both NCCLS incubation times MICs (98–99%). Although MICs for 10 of the 20 *C. lusitaniae* isolates could not be obtained at 24-h (lack of colour change) by the YeasOne plate; the comparison of 48-h MICs was excellent for both agents.
Conclusion: These data suggest the potential value of the YeastOne plate for use in the clinical laboratory to test the susceptibilities of clinical *Candida* spp. isolates to voriconazole.

O401 Susceptibility of *Aspergillus fumigatus* to voriconazole by flow cytometry

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The reference method for susceptibility testing of moulds NCCLS M38-A is cumbersome and time-consuming. Flow cytometry (FC) has proved to be a valuable alternative for susceptibility testing of *Candida* spp (1) and it would be of interest to evaluate its role in susceptibility testing of moulds.
Objective: To study the antifungal activity of Vor on clinical isolates of *A. fumigatus*, using FC with the probes FUN-1 (a marker of metabolic integrity) and propidium iodide (PI) (a marker of cell death by cell membrane lesion).
Methods: Two clinical strains of *A. fumigatus* were employed. MIC values were determined according the NCCLS M38-A protocol. Conidial suspensions (2×106 conidia/mL) were incubated at 37°C with different concentrations of Vor (MIC, $2 \times$ MIC, $4 \times$ MIC, MIC/2, MIC/4), for 8 h. Conidia were washed (to prevent interference between antifungal agents and the probe), suspended in PBS with 2% glucose and stained with 5 μg/mL of FUN-1 or 1 μg/mL of PI, for 30 min. Heat-treated conidia (90°C, for 30 min) were used as the control for cell death. The conidial cells were analysed by FC (Beckman-Coulter Corp., Hialeah, FL, USA): the morphology (scattergram) and the intensity of fluorescence of the stained cells (FL2 (green) for FUN-1and FL3 (red) for PI) were determined.
Results: It was possible to establish a MIC for Vor after 4 h by FC. At this time, virtually all conidia were metabolically impaired, as shown by FUN-1 staining. Similar cytometric results were seen with $2 \times$ MIC and $4 \times$ MIC. Dead conidia were detected with PI staining after 5 h, at MIC concentration.
Conclusions: FC can be used to determine the susceptibility of *A. fumigatus* to Vor. FC is a rapid and reliable alternative to the standard NCCLS M-38A method. Moreover, FC provides key information regarding the mechanism of antifungal activity of Vor and allows the analysis of a large amount of conidia.

References

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O402 *In vitro* activity of novispirins-antimicrobial peptides with broad spectrum activity

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Objectives: Antimicrobial peptides (AMPs) are ubiquitous in nature where they establish a first line of defence against invading pathogens or competing microorganism. A series of small α-helical peptides (NZ1000) are being investigated for potential therapeutic use and have antimicrobial activity against both Gram-positive and Gram-negative bacteria. A lead peptide from this series novispirin (NZ1001) has been tested in several topical and systemic animal models. In addition, several variants of NZ1001 have been generated. We report here the antimicrobial activity of novispirin and its variants.
Methods: Antimicrobial activity and spectrum of the purified compounds were assessed by determining the minimum inhibitory and bactericidal concentrations using the NCCLS guidelines with the addition of 0.01% BSA. Kinetics of bactericidal activity was characterized by time kill experiments at several concentrations.
Results: Purified novispirin showed potent bactercidal activity against both Gram-positive and Gram-negative bacteria including drug-resistant strains with MICs and MBCs as low as 0.25 μg/mL against *Shigella dysenteriae*. In general, the novispirins are more potent against Gram-negative organisms with MICs and MBCs of 0.25–8 μg/ml. In addition, novispirins do not show any cross resistance with traditional antibiotics when tested against drug-resistant strains including several clinical isolates. Analysis of the killing kinetics against *Escherichia coli* revealed that novispirins eliminate >99.9% of the bacteria in <30 min while against *Staphylococcus carnosus* the same level killing is observed after 3 h. Several antibiotics including vancomycin, tetracycline, penicillin, erythromycin and gentamicin were also included in the time kill studies and the novispirins display a more rapid killing than the control antibiotics.
Conclusion: Novispirin and its variants demonstrate potent cidal activity against both Gram-positive and Gram-negative bacteria including several drug-resistant strains. In addition, novispirins kill bacteria faster than traditional antibiotics.

O403 Study of the impact of macrolides against *Pseudomonas aeruginosa* cystic fibrosis isolates grown as biofilm

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Pseudomonas aeruginosa lung infection is the most important factor of mortality and morbidity among cystic fibrosis patients. While planktonic model is generally associated with inital steps of the pathology, bacteria grown as biofilm are pathognomonic of the chronic phase of infection. This last is responsible for deleterious effects but little is known about antibiotic susceptibility.
Objective: To know the impact of macrolides against *P. aeruginosa* strains grown both as planktonic and biofilm models.
Methods: Azithromycine and clarithromycine alone or associated with ceftazidime, tobramycine, doxycycline and sulfamethoxazole were tested against six cystic fibrosis clinical isolates (four smooth and two mucoid strains) and bactericidal kinetics were evaluated in a planktonic model. Biofilm formation in presence of four sub-inhibitory concentrations of the macrolides mentioned above was then followed-up.
Results: In planktonic model, tobramycine and ceftazidime could be fully bactericidal for concentrations as low as 2 mg/L after 2 h of incubation. When combined with ceftazidime, macrolides led to a prolonged bactericidal effect up to 24 h. Interestingly, doxycycline led to a significant reduction of the inoculum after 6 h for 75% of the strains tested. Furthermore, the combination macrolide-doxycycline enhanced this reduction. When biofilm formation was followed-up, a reduction of adhesion could be observed and interestingly, a significant impairment of colonisation resulting in a delayed biofilm structure could be noted when azithromycine was used. This effect was concentration-dependent and was maximal between 0 and 20 h (three log reduction vs. control).
Conclusion: These results indicate that macrolides, essentially used in cystic fibrosis therapeutics for their anti-inflammatory properties could enhance the bactericidal effects of other antibiotics in the initial steps of colonisation. Furthermore azithromycine seems to interfere not only with bacterial adhesion but also with factors involved in the initial phases of biofilm formation. Further tests including macrolides associated with other antibiotics are actually performed and should provide new therapeutical approach.

O404 Comparison of the mutant prevention concentration of gatifloxacin, garenoxacin, gemifloxacin, levofloxacin and moxifloxacin against clinical isolates of *Streptococcus pneumoniae* collected prior to (1994–1997) and after 1997 (1997–2003)

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Objectives: SP is an important human pathogen and antimicrobial resistance has compromised the use of many agents. Gatifloxacin (GA), garenoxacin (GAR), gemifloxacin (GM) and moxifloxacin (M) have enhanced *in vitro* activity against *Streptococcus pneumoniae* (SP) [compared with levofloacin (L)]. The mutant prevention concentration (MPC) defines the drug concentration threshold that prevents the growth of first-step mutants. New quinolones (Q) were previously shown to be less like to select for resistance than L by MPC and we speculated that use of less-active agents would elevate minimal inhibitory concentrations (MIC), MPCs or both. We tested SP isolates collected prior to (1994–1996) and after 1997 (1997–2003) by MIC and MPC.
Methods: MIC testing was by microbroth dilution in Todd–Hewitt broth and in accordance with NCCLS guidelines. For MPC testing approximately 10(10)cells were applied to blood agar plates containing drug, incubated at 35–37°C in 5% CO_2 and screened for growth at 24 and 48 h. MPC was the lowest drug concentration with no growth.
Results: Over >400 isolates were tested (1994–1996, 106; 1997 to present, >300). MIC distributions for SP remained unchanged during both time periods. MPC90 (mg/L) values remained unchanged during both periods respectively except for GAR (0.125 vs. 0.25) and GM (0.25 vs. 0.5). 2.5% of isolates from 1994–1996 had MICs to L ≥2 mg/L compared with 6% from 1997 to present. MPC90 values to L were 4 mg/L and <1% of isolates from 1994 to 1996 had L MPCs ≥8 mg/L when compared with 7% of isolates from 1997 to present. Rank order of potency based on MPC90 values (μg/mL) was GR 0.25 > GM 0.5 >M 1 > GA 2 > L 4.
Conclusion: The percentage of organism with MICs and MPCs to L was higher but not for other compounds for isolates collected from 1997 onward. The MPC concept predicts that the use of less-active compounds for SP may increase the likelihood of resistant SP. L was the least active agent on this study. Preferential use of Qs more active against SP may slow the rate of resistance emergence.

O405 Virulence factors of ciprofloxacin-resistant *Escherichia coli* in intensive care units

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Objectives: Extraintestinal-pathogenic strains (ExPEC) of patients in intensive care units (ICU) belong disproportionately to the virulence-associated group B2, in contrast to ciprofloxacin-resistant isolates (FQREC). A recent scrutiny of nine FQREC strains in regard of 35 VF has revealed a lower frequency of particular VF. Hence a lower virulence-potential of FQREC was concluded (*J. Infect. Dis.* **186**: 1852). The aim of this study was to investigate by use of a larger number of ExPEC whether the FQREC of the

four phylogenetic groups are distinct from ciprofloxacin-susceptible isolates (FQSEC) in regard of occurrence and distribution of VF and whether this is only related to the various frequencies of the different virulent groups.
Methods: In order to examine a large and comparable number of both FQREC and FQSEC isolates from the same groups, 137 isolates were selected from a collection of *E. coli* of known group assignation originating from 28 ICU. PCR was used to determine the presence of seven VF: the siderophores aerobactin (iutA) and yersiniabactin (fyuA), the capsule synthesis-gene locus (kpsMTII), the invasiveness factor (ibeA), the serum-resistance factor (traT), the adhesins (papA, papG) and the iron-regulated gene homologue adhesin (iha). The numbers of detected VF in one isolate were added up to give an aggregated VF score.
Results: Comparison of both resistance types showed that FQREC revealed a larger number of VF than FQSEC with regard to the groups A (2.41 vs. 1.69), B2 (4.56 vs. 3.65) and D (4.00 vs. 3.07) and with the exception of group B1 (1.07 vs. 2.00). The frequencies of particular VF significantly differed between FQREC vs. FQSEC in the groups B1 (fyuA: 7.1% vs. 57.1%), B2 (papG: 11.1% vs. 64.7%; traT: 88.9% vs. 35.3%) and D (papA: 60% vs. 20%; papG: 56% vs. 13.3%; fyuA: 84% vs. 46.7%; traT: 44.0% vs. 80%; iha: 60% vs. 20%).
Conclusions: Analysis of FQREC and FQSEC, taking into consideration the phylogenetic groups, did not give any indication of a lowered virulence potential in resistant *E. coli* with the exception of group B1 strains. Contrarily, FQREC tend toward a larger number of VF than FQSEC. Furthermore, the comparison of both resistance types within the same phylogenetic group revealed an accumulation of particular VF in FQREC. This investigation contradicts a recently published assumption of a lowered virulence potential in ciprofloxacin-resistant *E. coli*.

O406 *In vitro* and *in vivo* fitness cost of antibiotic resistance in *Escherichia coli*

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Objectives: Little is known of the fitness cost that antibiotic resistance exerts on wild-type bacteria, especially in their natural environments. We therefore examined the fitness costs that four antibiotic resistance-encoding genetic elements (AbR element) exerted on a wild-type *E. coli* isolate.
Methods: A rifampin-resistant (RifR) derivative of *E. coli* 345-2, a recent porcine isolate, was selected as the study organism based on its ability to compete successfully *in vitro* and *in vivo*. Plasmid R46, transposons Tn3 and Tn7 and a K43R RpsL substitution were separately introduced into 345-2 RifR using methods mirroring those that may occur in the wild. The insertion sites of Tn3 and Tn7 were identified using PCR and DNA sequencing. The fitness cost of each AbR element was assessed *in vitro* by pairwise growth competition in Davis minimal medium against the isogenic 345-2 RifR parent and *in vivo* by monitoring the number of CFU/g of faeces regularly for 21 days following inoculation of six 7-week-old organic piglets with 109 CFU. The organism was recovered by plating onto MacConkey agar containing rifampicin and retention of the AbR elements was monitored by replicating onto agar containg the appropriate antibiotics. Each derivative of 345-2 RifR carrying an AbR element was grown in laboratory culture without antibiotics for 200 generations in an attempt to eliminate any fitness costs associated with carriage of AbR elements and the experiments to assess fitness were repeated.
Results: Tn3 had inserted into a cryptic DNA sequence of unknown location. Tn7 had inserted downstream of glmS, as expected. R46 and RpsL K43R were found to impose fitness costs on *E. coli* 345-2 RifR *in vitro* but did not compromise survival *in vivo*; on the contrary the strain carrying R46 that had been grown for 200 generations *in vitro* appeared to survive better *in vivo* (Table 1) than the AbR-free parent. Acqusition of Tn7 had no impact on the fitness of *E. coli* 345-2 RifR *in vitro* or *in vivo* (Table 1). Acquisition of Tn3 improved fitness of *E. coli* 345-2 RifR *in vitro* and *in vivo* (Table 1).

Table 1. Fitness impact of Ab^R elements on *E.coli* 345-2 Rif^R

Ab^Relement	*In vitro*[a]	*In vitro* after 200 generations growth *in vitro*[a]	*In vivo*[b]	*In vivo* after 200 generations growth *in vitro*[b]
None	NA	NA	50(10–110)	NA
R46 plasmid	−3.3 ± 1.7	−3.8 ± 1.6	20(0–60)[c]	1270(0–4900)
RpsL K43R	−2.2 ± 0.9	−3.6 ± 0.9	120(10–510)	40(10–50)
Tn3	+6.0 ± 2.6	+3.4 ± 1.3	380(10–500)	4430(80–5130)
Tn7	+1.2 ± 1.2	+0.8 ± 4.4	20(0–40)	110(0–420)

[a]Per generation fitness impact on *E.coli* 345-2 Rif^R(%)
[b]Mean CFU/g of faeces (range) on day 21 post-inoculation
[c]value for day 17 as no isolates were recovered on day 21. NA(not applicable)

Conclusions: The fitness impact exerted upon *E. coli* 345-2 RifR by carriage of AbR elements is variable depending on the element. Costs tended to be lower *in vivo* than *in vitro*. In most cases *in vitro* passage for 200 generations did not affect fitness. It is unknown whether the fitness gain brought about by Tn3 acquisition was a function of the insertion site or the transposon itself.

O407 Fitness cost of *sul2*-coding plasmids in *Escherichia coli*

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Objectives: Following national advice to prescribe trimethoprim instead of trimethoprim-sulphamethoxazole there has been a 97% reduction in sulphonamide use in the UK since 1991. Nevertheless, the prevalence of resistance among clinical *E. coli* isolates remains at approximately 40–45%. Linkage of sulphonamide resistance to other resistances is thought important for this maintenance, but it also implies that sulphonamide resistance exerts little fitness cost. We therefore examined fitness costs of three plasmids carrying the commoner sulphonamide resistance determinant, *sul2*.
Methods: The per-generation fitness costs of three *sul2* plasmids were determined by pairwise growth competition against isogenic plasmid-free bacteria in Davis minimal medium. The sequence of plasmid p9123 was determined by primer walking.
Results: Two of three *sul2* plasmids imposed fitness costs, whereas p9123 improved the fitness of both its original clinical host and *E. coli* JM109 (Table 1) by approximately 4%. Sequencing revealed that p9123 carried the remnants of a transposon-like structure containing the *sul2*, *strA* and *strB* genes. Outside this structure p9123 encoded four putative open reading frames of unknown function.

Table 1. Fitness impact of plasmids coding for *sul2*

Plasmid	Size(kb)	Resistance Phenotype	Per generation fitness impact on clinical *E. coli* host (%)	Per generation fitness impact on *E. coli* JM109 (%)
p9123	6.2	Su	+4.3 ± 1.9	+4.1 ± 2.8
p9938	6.7	Su Tm	−18.2 ± 15.4	−9.0 ± 1.5
p9118	>66	Su S A	NA	−4.7 ±3.1

A(ampicillin), C(chloramphenicol), T(tetracycline), S(streptomycin) Tm(trimethoprim), NA(not applicable as plasmid could not be cured)

Conclusions: The fitness advantage conferred on their hosts by some *sul2* plasmids such as p9123 may have contributed to the maintenance of sulphonamide resistance in the UK in the absence of clinical selection pressure. The mechanism by which p9123 confers a fitness advantage on its host is unknown. Other *sul2*-coding plasmids remain prevalent despite their fitness costs, probably due to co-selection by other agents. These data suggest that once antibiotic resistance has been established on mobile genetic elements, it may be difficult to eliminate.

O408 High prevalence and combinations of antimicrobial resistance traits in commensal bacteria from a very remote rural community of Alto Amazonas (Peru)

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Objectives: Several aspects concerning the impact of antimicrobial usage in the emergence and spread of resistance remain poorly understood and are difficult to investigate due to the almost universal use of antimicrobial agents for several decades, and to the lack of representative bacterial collections from the preantibiotic era. The purpose of this work was to investigate antimicrobial susceptibility of the commensal *Escherichia coli* of the population of a very remote rural community of Peruvian Amazonas, where the use of antimicrobials has been minimal.
Methods: The studied community (113 subjects) was selected as one of the most isolate of the Alto Amazonas Province. Antimicrobial susceptibility of the commensal *E. coli* was investigated by means of a rapid screening method in which faecal swabs were plated onto McConkey Agar and antimicrobial disks (ampicillin-AMP, ceftriaxone-CRO, tetracycline-TE, chloramphenicol-C, trimethoprim-sulphamethoxazole-SXT, quinolones and aminoglycosides) were applied onto the seeded plate. Resistant isolates showing an *E. coli*-like colony morphology were collected, identified, and the susceptibility pattern was then confirmed by standard disk-diffusion method. A simple questionnaire was used to investigate antimicrobial usage (last 12 months) and to collect demographic information.
Results: A total of 93 healthy subjects (42 males, 51 females; age range 0–59 years, median 9) was enrolled. Five of them (5%) reported previous antimicrobial use. Eighty-three of 93 (89%) subjects carried commensal *E. coli* resistant to at least one antimicrobial agent. The highest resistance rates were observed for TE (82%), AMP (68%), SXT (65%), and C (44%). The majority (85%) yielding antibiotic-resistant *E. coli* harboured multidrug–resistance (MDR) strains. The most frequent MDR phenotype included AMP, TE, and SXT. No resistance was detected against ceftriaxone, amikacin, nalidixic acid and ciprofloxacin.
Conclusion: The high resistance rates unexpectedly detected in this isolate community suggest that, in some cases, the spread and maintenance of resistant strains and resistance determinants could be not directly related to antimicrobial consumption. The notable diversity of resistance traits and of their combination patterns, underscore a considerable complexity of the resistant bacterial ecosystem and of their resistance-associated metagenome.

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O409 Bacterial fitness of drug-resistant *Pseudomonas aeruginosa*

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Study objective: To investigate the properties of drug-resistant *Pseudomonas aeruginosa* mutants regarding bacterial fitness, i.e. their survival in the environment.
Materials and methods: Development of resistance against cefepime, imipenem, meropenem and gatifloxacin was investigated in clinical isolates of *P. aeruginosa* $n = 10$) in up to 30 serial passages on microtitre plates containing concentrations from three steps below to at least four steps above the MIC of the respective antibiotic. Antimicrobial resistance was defined as a four-step (16-fold) increase in the MIC with stability after 10 serial passages on antibiotic-free media. Environmental behaviour of the drug-resistant mutants selected *in vitro* was compared with the isogenic parental strains The survival of the bacteria was tested in (i) sterile tap water and (ii) on a dry surface (patapar cellulose) at room temperature (22°C). Survival of *P. aeruginosa* in water was analysed once daily for a study period of 2 weeks, whereas survival on the dry surface was investigated 2, 4, 6, and 8 h after inoculation. Tests in water were performed in duplicate, tests on the dry surface in triplicate.
Results: Of all the *P. aeruginosa* mutants tested in water ($n = 40$), five (12.5%) showed significantly improved survival in tap water when compared with their isogenic parental strain, whereas 10 (25%) mutants showed significantly impaired growth ($P < 0.05$); the remaining 25 (50%) mutants did not show any differences in growth kinetics. Improved growth kinetics were observed among cefepime and gatifloxacin-resistant mutants. Impaired bacterial fitness was noted in half (50%) of the meropenem-resistant and a third (30%) of the imipenem-resistant mutants. On a dry surface, four *P. aeruginosa* mutants (10 %) showed improved growth, only one mutant (2.5%) showed impaired fitness. There was no difference in growth kinetics among the remaining 35 mutants (87.5 %).
Conclusions: Impaired bacterial fitness in multidrug-resistant *P. aeruginosa* is not a rare phenomenon. However, bacterial fitness can vary according to environmental conditions. In our *in vitro* study, bacterial fitness was particularly impaired among carbapenem-resistant strains, whereas in some cases, an increase in bacterial fitness occurred in cefepime and gatifloxacin-resistant mutants. The importance of these findings deserves further study.

Mycobacterial infections: diagnosis and epidemiology

O410 Increased expression of adhesion molecules on peripheral blood monocytes in patients with severe pulmonary tuberculosis

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Objectives: The aim of the present study was to determine whether the expression of monocyte adhesion molecules (MAM) on peripheral blood monocytes from patients with active pulmonary tuberculosis (TB) is increased compared with healthy volunteers or to other respiratory infections (ORI).
Methods: Blood samples were obtained from 88 patients with active pulmonary TB at admission, and also after 4 and 8 weeks in severe cases ($n = 53$). A group of healthy volunteers ($n = 53$) and an ORI group ORI ($n = 34$) were also investigated. Blood was incubated with FITC-labelled monoclonal antibodies against CD11a, CD11b, CD11c, CD35, CD54, CD49a, CD58. Fluorescence

intensity was measured by flow-cytometry. Serum levels of ICAM-1, VCAM-1 and IL-8 were measured by an enzyme linked immunoassay.
Results: No differences in the expression of MAM could be observed when comparing patients with mild to moderate TB vs. ORI patients or healthy volunteers. In contrast, severe TB patients presented a significantly higher expression of all MAM ($P < 0.0001$, except for CD49a) when compared with healthy volunteers, and higher levels of CD11a, CD11c, CD35, CD54, CD58, ICAM-1 and VCAM-1 when compared with ORI patients ($P < 0.04$). No decrease was noted in MAM levels from severe TB patients during the follow-up period.
Conclusion: Severe pulmonary TB is associated with an increased expression of the monocyte adhesion molecules compared with healthy volunteers or to patients with other respiratory infections. These levels are maintained over 8 weeks, reflecting a persistent process of recruitment of monocytes into the lung tissue despite antituberculotic therapy.

O411 The diagnostic potential of the QuantiFERON-RD1 test in patients with active tuberculosis

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Objectives: The QuantiFERON(QFN) test may have the potential to replace the tuberculin skin test and it has been launched for immuno-diagnosis of latent tuberculosis infection (LTBI) in low risk population. The aim of this study was to evaluate potential of the QFN test improved by the two *M. tuberculosis* specific antigens (CFP-10 and ESAT-6) for the diagnosis of active TB.
Methods: We have measured IFN-γ responses to the *M. tuberculosis*-specific RD1 coded antigens, CFP-10 and ESAT-6 in a whole blood assay (QFN-RD1) and compared the sensitivity with conventional microscopy and culture (M/C). Prospectively, 82 patients with high risk of TB were enrolled; 51 of these suffered from active TB and 31 patients did not have TB. Twenty-three patients with unrelated diseases and 39 healthy persons were included as controls. Whole blood samples from all donors were analysed using the QFN-RD1 without knowing the final diagnosis of the patient. ROC curves analysis based on data from 39 healthy donors was used to select the cut-off.
Results: The QFN-RD1 was positive in 82% (42/51) of the TB patients and was negative in all 39 healthy individuals. Under these conditions sensitivity was 82% and specificity was 100%. More patients were diagnosed by the QFN-RD1 (82%) than by M/C 62% (32/51, $P = 0.043$) and a high percentage 89% (17/19) of smear-negative TB patients were QFN-RD1 positive. Interestingly, 92% (12/13) of the patients with extra-pulmonary TB were QFN-RD1 positive, whereas only 39% (5/13) were positive by M/C ($P < 0.02$). Among patients with no TB suspicion, the proportion of QFN-RD1 responders was low 17% (four of 23) whereas 40% (10/25) of the patients with a strong clinical suspicion of TB had a positive response. The majority of the latter patients had one or more risk factors (born in high endemic country, alcohol misuse, or IVDU), and we suggest that these QFN-RD1 positive patients without active TB had a LTBI.
Conclusion: The sensitivity of the QFN-RD1 was high especially in cases extra-pulmonary TB, and the QFN-RD1 may serve as an additional diagnostic tool especially in situations where material is not readily available for M/C. None of the healthy unexposed individuals responded indicating high specificity, but 40% of the patients with high risk of TB responded, which strongly suggests LTBI. The impact of latent TB on specificity in different settings remains to be determined and we suggest that all QFN-RD1-positive individuals should be follow clinically.

O412 The role of a new immune diagnostic test based on ESAT-6 epitopes in the evaluation of subjects with a clinical suspicion of active tuberculosis

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Background: *Mycobacterium tuberculosis* ESAT-6 purified protein and its peptides are currently being evaluated as antigens for the immune diagnosis of TB. We set up an ELISPOT assay for IFN-γ whose novelty consists on two multiepitopic peptides from ESAT-6 protein, selected by computational analysis, that allows the discrimination between latent and active TB and the monitoring of the efficacy of anti-TB therapy (Vincenti *et al.*, *Mol. Med.* 2003; Carrara *et al.*, *Clin. Infec. Dis.*).
Objective and methods: Aim of this study was to assess the performance of the ELISPOT assay based on our selected ESAT-6 peptides in the diagnosis of TB. In 12 months, a total of 120 HIV-negative patients and 61 HIV-positive patients with symptoms and signs consistent with active pulmonary or extra-pulmonary TB were prospectively enrolled. *In vitro* IFN-γ response to ESAT-6 intact protein and its selected peptides, PPD, recall antigens and mitogens was evaluated in PBMC by ELISPOT assay.
Results: Of the HIV-negative patients enrolled, 67 (56%) were diagnosed with TB and in 53 of them the diagnosis was microbiologically confirmed (culture from biological fluids and/or PCR from biopsies specimens). Among the HIV-positive patients, 32 (52%) were diagnosed with microbiologically confirmed TB. *In vitro* anergy (unresponsiveness to mitogen) was present in 10% of HIV-negative patients vs. 25% of those with HIV disease. The analysis was performed among the nonanergic patients. In particular, among the HIV-negative patients, 44/62 with TB and four of 49 without TB had a positive response to ESAT-6 selected peptides. Thus, the sensitivity of our assay in this group is 71%, specificity is 91%, positive and negative predictive values are 92% and 71%, respectively. Among HIV-positive patients, 17/23 with TB and one of 23 without TB had a positive response to ESAT-6 selected peptides. Thus, sensitivity of our assay in this group is 70%, specificity is 93%, positive and negative predictive values are 95 and 73%, respectively. In contrast, in both groups of patients with or without HIV-disease, an *in vitro* response to PPD and the intact ESAT-6 protein did not allow a discrimination between subjects with active TB compared with those without.
Conclusions: This immune diagnostic assay, although with a still sub-optimal sensitivity, has a high positive predictive value for active TB, providing rapid (2 days) and specific diagnostic information in patients with or without HIV disease.

O413 Evaluation of a new automated, rapid, colorimetric culture system using solid medium for laboratory diagnosis of tuberculosis and determination of antituberculosis drug susceptibility

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Objective: To evaluate the performance of Dio-TK Culture System (CS), a new colorimetric, automated and rapid CS.
Methods: Results of Dio-TK CS were compared with routinely used Löwenstein Jensen (LJ) medium and BACTEC 460 TB CS.
Results: In this study, 449 specimens, mostly sputum samples obtained from 348 patients were evaluated. *Mycobacteria* isolated from 31 (6.9%), 23 (5.1%), 18 (4.0%) and 21 (4.7%) of the specimens in BACTEC 12B, LJ, Dio-TK Medium and Dio-TK SLC (selective), respectively. The mean time to detection of growth of 13 isolates by BACTEC 12B, Dio-TK Medium, Dio-TK SLC and LJ medium were 8.9, 15.1, 17.0 and 26.1 days, respectively. Contamination ratios for BACTEC 12B, Dio-TK Medium, Dio-TK SLC and LJ media were 1.3% (*n*: 6), 3.8% (*n*: 17), 1.6% (*n*: 7) and 2.4% (*n*: 11), respectively.

Conclusion: Dio-TK CS may be a practical and rapid CS for daily use. However, the manufacturer should improve the CS to minimize the effects of manipulation errors. Comparative studies with a larger number of isolates are needed to standardise drug concentrations used in antituberculosis drug-susceptibility testing.

O414 Quality assurance in molecular diagnosis of tuberculosis

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Objectives: The analysis of laboratory data and their correlation with clinical information constitutes an important quality assurance exercise. We retrospectively analysed all data on molecular diagnosis of tuberculosis recorded in a laboratory, which started functioning as a diagnostic center since April 2000.
Methods: Laboratory data (specimen type, volume and number/patient; PCR results) obtained from April 2000 to October 2003 were analysed, while clinical and other laboratory findings were recorded for patients submitting specimens during the last 13 months, and for all patients with PCR(+) specimens. Two diagnostic PCR methods were applied, a screening and a confirmatory assay.
Results: During a 42-month period, 508 samples from 291 patients originating from 35 hospitals were examined. Sputum ($n = 92$, 18%), urine ($n = 65$, 13%) and CSF ($n = 55$, 11%) were the most frequent samples, while 174 (34%) originated from the lower respiratory tract. Mean volume for CSF samples was 1.8 mL, for pleural fluid 24.5 mL and for peritoneal fluid 45.8 mL. Of 39 patients submitting sputum samples, 24 demonstrated three consecutive samples (62%) and four patients two samples (10%), while three consecutive urine samples were submitted from 18 of a total 28 patients (64%). Ten patients revealed at least 1 PCR(+) sample: eight demonstrated positive cultures, one was culture-negative and for one, cultures were not performed. PCR testing was negative for seven patients for whom tuberculosis diagnosis was established either by histology or clinical criteria but not cultures (three pleural fluids, two cutaneous biopsies, two biopsies in paraffin). During the last 13 months, 123 specimens from 91 patients were examined. Mycobacterial cultures were submitted for 57 patients (62%), were not submitted for 19 (21%), while culture status was unknown for 15 (17%). Specificity and PPV for PCR were 100%, NPV 91% and sensitivity ranged from 100% (lower respiratory tract specimens) to 0% (tissue in paraffin and pleural fluid).
Conclusions: Better communication between laboratory and clinicians will improve specimen quality in terms of adequate number and volume of specimens and ascertain that they are all cultured. The high frequency of extrapulmonary samples reflects the necessity to rapidly rule out/in extrapulmonary tuberculosis, for which, the sensitivity of molecular diagnosis is compromised. The application of quality assurance methods can only improve patient care.

O415 Molecular characterisation of *Mycobacterium tuberculosis* strains from Southern Ukraine: evidence of drug resistance rates and Beijing strains prevalence

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High rates of TB and HIV incidence were observed recently in Ukraine, particularly in the Southern region.
Objective: Detection of rifampicin, isoniazid, multidrug resistance rates and Beijing strain prevalence in TB isolates from Odessa and Nikolaev oblasts (southern Ukraine) in 2003 using a macroarray technique and spoligotyping.
Materials and methods: In total, 110 *Mycobacterium tuberculosis* strains isolated from patients of TB hospitals in Odessa and Nikolaev oblasts (both chronic and new cases) were available for study. The macroarray method is based on amplification of *rpoB*, *katG* and *inhA* genes fragments with three pairs of biotin-labelled primers followed by reverse hybridisation of PCR products with a set of oligonucleotide probes immobilized on nylon membranes. DNA extracts were prepared by heating cell suspensions followed by chloroform deproteinisation. Streptavidin-alkaline phosphatase colour development system was used for results visualisation. Spoligotyping was done using standard protocols.
Results and discussion: In total, 32.7% of isolates possessed mutations consistent with rifampicin resistance, 41.8% consistent with isoniazid resistance and 29.1% possessed mutations both in *rpoB* and *katG* (or *inhA*), i.e. were considered to be multidrug resistant (MDR). In strains isolated from the patients who had never received TB treatment MDR rates did not exceed 16%. Analysis of occurrence of different types of mutations consistent with drug resistance development demonstrated that in the majority of cases rifampicin resistance was due to mutations in codon 531 (54.8% of all resistant isolates) and in codon 526 (25.8%) of *rpoB* gene. 76.0% of isoniazid resistant isolates possessed mutations in codon 315 of *katG* gene, and other 24.0% both in *katG* and *inhA* genes. Spoligotype patterns were obtained for all strains, 30 (27.3%) of which produced a typical Beijing family profile (hybridisation with final nine probes). According to these results, *M. tuberculosis* drug resistance rates for the southern Ukraine are lower than in north-western and central Russia, but still much higher than in the majority of European countries. A relatively low prevalence of Beijing family strains and a wide range of mutations associated with drug resistance demonstrate genetic diversity of *M. tuberculosis* strains circulating in southern Ukraine although this may not reflect epidemiological situation in the Ukraine as a whole.

O416 Repetitive element typing of *M. tuberculosis* from the Caribbean and African population in the UK reveals unsuspected transmission

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Objectives: Tuberculosis case rates are increasing worldwide. In Birmingham in recent years, case numbers have risen in common with other metropolitan areas in the UK. Different molecular typing methods are being developed and have been used to examine strains from clusters, and broader population studies. VNTR and MIRU are PCR-based methods that give an easily reproducible, numerical output. The combination of the two methods has been shown to be highly discriminatory and useful for outbreak investigation. Spoligotyping is a reproducible PCR-based method with simple binary output. Plans are under development to introduce real time molecular typing into routine clinical practice. Will this add information to that collected by routine contact tracing and which method will give the most useful results?
Methods: DNA was extracted from *M. tuberculosis* cultured from 101 patients in Birmingham with tuberculosis, from two distinct ethnic groups, during the years 1999–2002. The DNA was analysed by VNTR and spoligotyping. Those strains with identical VNTR profiles were further analysed by MIRU typing.
Results: Spoligotyping identified 11 clusters with 59 clustered isolates. African and Caribbean isolates were mainly separated in different spoligotyping clusters. MIRU typing reduced the number of clusters to seven, with 38 clustered isolates. The larger spoligotyping and VNTR clusters were sub-divided by MIRU typing and three MIRU clusters were split by spoligotyping, with the loss of a single spacer. There was a group of 15 Caribbean patients with identical MIRU profiles, not previously identified as epidemiologically linked by traditional contact tracing. This may explain the on-going excess rates of tuberculosis within this community.

Most of the Caribbean population were born in the UK and thought to have acquired their infection locally. 67% of VNTR

types were clustered, the Harlem clade, 32333, being most commonly represented. The newly identified X clade, 32 433, was seen in seven patients, five of whom were born in the Caribbean. In contrast, most of the African populations were recent arrivals in the UK and had disease as a result of re-activation of latent infection rather than from local spread. Only 27% of VNTR types were clustered.
Conclusion: A combination of VNTR and MIRU typing is more discriminatory than spoligotyping and can identify unsuspected community transmission of tuberculosis and aid work to control spread of disease.

O417 Clinical and social profile of TB patients in civilian and penitentiary sectors in Samara, Russia

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Background: The epidemics of tuberculosis (TB) and HIV are continuing in the Russian Federation particularly within the prison system.
Design: Cross-sectional study of TB patients with and without HIV co-infection in the prison and civilian sectors of Samara, Russia Federation.
Objectives: Describe the demographic, clinical, social and HIV status of civilian and penitentiary TB patients.
Results: Individuals (3408) with TB were enrolled including 1345 prisoners. The majority of civilians were male (74.7%); 20.8% had been in prison before. Many had advanced disease: approximately 30–40% of patients had two or more zones and/or cavities on CXR; 89.9% had been BCG vaccinated. For all TB cases HIV co-infection was seen in 4.7, 2.4 and 7.5%, respectively; hepatitis B/C co-infection in 18.2, 14.1, and 24.3%; previous gonorrhoea was seen in 4.7, 3.6 and 6.4%. Two-thirds (66.1%; 84/127) of the HIV-positive patients were IVDU. Overall 726/3400 (21.4%) admitted to recreational drug use typically opiates (84.8%) by injection (87.8%). Male civilians compared with male prisoners with TB were less likely to be HIV-positive (OR = 0.07), co-infected with hepatitis B/C (OR = 0.59), use drugs (OR = 0.04), have had previous gonorrhoea (OR = 0.68), syphilis (OR = 0.3), to smoke (OR = 0.11) or drink alcohol (0.53). In the multivariate analysis, civilians were more likely to have COPD (OR = 28.75), localised disease on CXR (OR = 2.03), thoracic surgery (OR = 8.41), significantly less likely to have a productive cough (OR = 0.53), chest pain (OR = 0.33), be BCG vaccinated (OR = 0.09), have had syphilis (OR = 0.25) or herpes (OR = 0.03). They were also more likely to smoke (OR = 0.25) and to use recreational drugs (OR = 0.04). Prisoners with HIV and TB were more likely to have been treated for TB before (OR = 1.44), be on post-treatment anti-relapse therapy (OR = 2.61), and have jaundice (OR = 6.38) and concurrent hepatitis B/C (OR = 2.13) than those who were HIV negative. Multivariate analysis showed that HIV-positive individuals were more likely to have been treated (OR = 2.17), to have chest pain (OR = 2.01), jaundice (OR = 8.48) and gonorrhoea previously (OR = 2.25) but were less likely to report weight loss (OR = 0.47), shortness of breath (OR = 0.42).
Conclusion: Major risk factors for the spread of HIV and drug resistant TB exist particularly in the prison sector and there are important social and gender differences underlying the epidemiology of HIV and TB.

O418 Clinical presentation and outcome of HIV-associated tuberculosis in the HAART era in Rome

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Objectives: To assess changes in clinical presentation and outcome of pulmonary tuberculosis (TB) diagnosed in HIV-infected patients in the time period preceding and following the widespread introduction in clinical practice of highly active antiretroviral therapy (HAART) in Italy.
Methods: We reviewed clinical charts of 221 HIV-infected patients with culture-confirmed pulmonary TB hospitalised at National Institute for Infectious Diseases ''L. Spallanzani'', Rome from January 1987 to December 2002. A total of 121 patients diagnosed in 1987–1996, i.e. before HAART introduction, were compared with 100 patients diagnosed in 1997–2002, i.e. after HAART introduction. Differences in categorical variables were analysed with the use of the chi squared test or Fisher's exact test, as appropriate.
Results: At onset, patients diagnosed in 1997–2002 were more likely to have TB as the first AIDS defining illness (79% vs. 56%, $P < 0.005$), to be foreign born (37% vs. 12%, $P < 0.005$), and had a higher CD4+ count (median 126 vs. 72/mm^3, $P < 0.005$). Moreover, post-HAART patients had more frequently a 'typical' chest X-ray pattern with upper-lobe infiltrate and/or cavitation (55% vs. 35%, $P < 0.005$), and *M. tuberculosis* resistant to at least both isoniazid and rifampin (12% vs. 5%; $P = 0.09$). We observed 20 cases of TB among post-HAART patients. By the end of the study period (September 30, 2003), 113 patients (51%) had died, 93 patients (42%) were alive and healthy, while 15 patients (7%) were lost to follow-up after TB diagnosis; 21 patients had died (21%) in post-HAART group and 92 patients (76%) in pre-HAART group ($P < 0.001$). TB was recorded as the cause of death in 34 patients (30%). The survival proportion at 1 and 3 years was 87% and 82% in patients diagnosed in the post-HAART era, while it was 59% and 26% respectively in pre-HAART patients. Univariate analysis showed that pre-HAART patients had a significantly decreased survival compared with the other patients ($P < 0.001$, log-rank).
Conclusions: We found that, after HAART became widely available, patients tended to be diagnosed with TB at lower level of immunosuppression, to have TB more frequently as the first AIDS defining illnesses, and to have more frequently a 'typical' chest X-ray pattern. Moreover, patients diagnosed in the HAART era had a significantly longer survival. Our data suggest that the increasing use of HAART has modified the clinical presentation of HIV-associated TB and the survival of patients presenting with this disease.

O419 Treatment of tuberculosis in HIV-infected patients

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Objective: To describe treatment practices and outcomes of tuberculosis and HIV infection in dually infection persons.
Methods: We retrospectively reviewed the medical records of all HIV-1 positive patients with TB, identified from December 1997 to October 2003 at the Infectious and Tropical Diseases Institute of the University of Brescia, Italy, collected on demographic parameters, viro-immunological status, TB site, treatment administered, response to treatment and side-effects and cases of AIDS-defining illness.

Results: A total of 42 HIV+ patients with tuberculosis were identified. Among them 59.5% were immigrants, 14.3% injection drug users; 54.1% were unaware of their HIV infection at the time of the TB diagnosis. All TB cases were classified as new cases and the diagnosis was based on isolation of *M. tuberculosis* or identification of acid-fast bacilli were detected on 91.3%. The 17.4% shown extrapulmonary involvement (23.9% TB lymphonode), 21.7% disseminated TB, 82.6% pulmonary (PTB) involvement with a smear positive in 45.6% of cases. Rifampin was administered in 93.5% of the cases, 95.3% of whom concluded the TB treatment; 6.5% were treated with rifabutine. The 43.5% of the cases developed the tuberculosis with a CD4 count <300/mm^3 L, and the 71.8% with a CD4 count <100/mm^3 L, according with the rate found of 21.7% of disseminated TB in patients with a low CD4 count. 76.1% of all TB and HIV co-infection cases received HAART; 62.8% of whom with NNRTI. During the follow-up, three patients developed an AIDS defining illness. Adverse events were recorded on 17.4% of all cases, none interrupt treatment because of side-effects. 4.7% failed, and 7.2% died. Comparing the CD4 cell count and HIV viral load at the beginning and end of TB treatment, we observed an increasing of CD4 cell count ($P = 0.07$) and reduction of the viral load (P, not significant).
Conclusions: 76.1% of dually infected patients in this study received HAART in combination with TB treatment. Side-effects occurred in 17.4%, but no one case interrupted HAART.

Infection in the immunocompromised host: diagnostic tools, treatment and monitoring

O420 A negative procalcitonin test as an indicator of noninfectious fever in neutropenic cancer patients with FUO

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Objectives: FUO accounts for 25–50% of all febrile episodes (FE) in neutropenic cancer patients and may reflect the presence either of a noninfectious fever or of an occult infection. Given that infection cannot be ruled out, most patients with FUO are treated with broad-spectrum antibiotics for extended periods of time (IDSA, CID, 2002). Identification of patients with NIF, in whom antibiotics could be stopped rapidly (i.e. within 48–72 h of fever onset), would help to reduce treatment costs, adverse events and minimise the risk of development of resistance. In children with meningitis and in critically ill patients, PCT has been shown to distinguish bacterial from viral infections or from inflammatory diseases. The aim of the present study was to evaluate the utility of PCT for the management of neutropenic FUO.
Methods: 171 FE in 125 consecutive neutropenic patients undergoing intensive chemotherapy for haematological malignancies were studied prospectively over 18 months. FE were classified as microbiologically (MDI) or clinically (CDI) documented infections and FUO according to standard definitions (ICHS, JID, 1990). Plasma concentrations of PCT (LUMItest®,BRAHMS, Germany) were measured twice weekly in the absence of fever and daily within 4 days of a FE test was considered to be negative if PCT was <0.5 ng/mL.
Results: Forty-six of 171 FE (27%) were classified as FUO. Among these, PCT remained negative in 32 (median 0.2 ng/mL) and became positive within 72 h in 14 (median 0.7) ($P < 0.001$) reaching levels comparable with those measured in MDI (median 0.8) and CDI (median 0.5). Apart from PCT levels at day 3, these two subgroups of FUO patients were clinically indistinguishable: peak temperature (38.5 vs. 38.6°C), time to defervescence (2 days, range 1–9 vs. 2 days, range 1–6) and duration of antibiotic therapy (8 days vs. 7 days). Thus, PCT was the only parameter capable of distinguishing FUO patients likely to have a NIF (low PCT) from those likely to have an occult bacterial infection (elevated PCT).
Conclusions: A negative PCT (<0.5 ng/mL) within 72 h after onset of fever in neutropenic cancer patients with FUO is an indicator of a noninfectious aetiology of fever suggesting that empirical antibiotic therapy might be discontinued in these patients. This strategy should be tested prospectively.

O421 Prevalence and impact of adenovirus viraemia in adult stem-cell transplant recipients treated with Campath-1H

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Objective: Determine the incidence and impact of ADV viraemia in adult recipients of peripheral or bone marrow stem-cell transplantation (PSCT, BMT) who received Campath-1H-based conditioning.
Methods: Whole blood and serum samples collected weekly were tested by PCR (Hexon gene). Stool, urine, nasopharyngeal aspirates and throat swabs were tested by PCR and culture (A549 cells) to assess virus excretion. Blood viral load kinetics were determined by real-time PCR.
Results: The study included 49 consecutive patients (30M, 19F; mean age 50 years, range 19–69) who received PSCT ($n = 34$) or BMT ($n = 15$) for haematological disease in May 2002–May 2003. Donor status was sibling HLA-identical ($n = 12$), unrelated (UD) HLA-identical ($n = 26$) and UD HLA-mismatched ($n = 11$). Over 748 person-weeks of follow-up and a mean follow-up to day 113+ (median 102+), ADV viraemia was detected in four patients. All had lymphocyte counts <0.25; two had failed a previous SCT, two others were recipients of UD HLA-mismatched grafts. All progressed to disseminated infection, with virus excretion at two or more sites, viral load >10 × 6 c/mL, and symptomatic disease (fever, gastroenteritis, pneumonia, haematuria or hepatitis). Two patients became viraemic at day 12+ (types C, A12) and died with multi-organ failure at day 15+ and 30+, respectively. A third patient became viraemic at day 26+ (type C) and reactivated CMV at day 33+. The ADV viral load declined during anti-CMV therapy with i.v. ganciclovir, but rebounded once therapy was discontinued. The patient died with multi-organ failure at day 76+. The fourth patient became viraemic at day 48+ (type E4)and progressed to disseminated infection with hepatitis. The viral load declined following the beginning of i.v. cidofovir at day 67+ and coinciding with lymphocyte count increasing >0.25. ADV PCR became negative in serum and subsequently in whole blood; virus excretion ceased at all sites and the patient made a full recovery by day 114+. ADV was detected in the stool of four additional patients in the absence of viraemia, but the infection resolved spontaneously.
Conclusions: In this cohort of heavily immunocompromised patients the incidence of ADV viraemia was 8.2%. Viraemia was

highly predictive of virus dissemination and morbidity and was associated with the entire mortality (three of 49) observed in the first 100 days post-SCT. Blood viral load dynamics correlated well with disease progression and response to antiviral therapy and/or immune reconstitution.

O422 Efficacy of caspofungin against invasive *Candida* or *Aspergillus* infections in neutropenic patients: review of the caspofungin database

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Objectives: Neutropenia is an indicator of poor prognosis in patients (pts) with fungal infections. *In vivo* models demonstrate that the echinocandin caspofungin (CAS) is effective against both *Candida* and *Aspergillus* infections even in persistently neutropenic animals. We reviewed the current Merck database to ascertain the efficacy of CAS in neutropenic pts with documented invasive aspergillosis (IA) or invasive candidiasis (IC).
Methods: This review was limited to neutropenic pts with proven/probable IA or IC at study entry. Data are available from four clinical trials: the salvage IA study (Protocol 019, P019), the comparative IC study (P014), the IA/IC compassionate use study (P024), and the empirical therapy (Rx) study in febrile neutropenic pts (P026). P026 included a subset of pts who had IC or IA based on clinical/radiology evidence of infection at study entry but in whom the diagnosis was not confirmed until after CAS was initiated. All pts had an ANC $<500/mm^3$ at CAS onset. In all pts, CAS was administered as monotherapy at 50 mg/day following a 70-mg loading dose on day 1. CAS was administered as salvage Rx in P019 and P024, and as first-line Rx in P014 and P026. Efficacy was assessed at the end of CAS Rx. Success included complete or partial responses.
Results: A total of 75 neutropenic pts were identified with proven/probable invasive infection, including 29 pts with IC and 46 pts with IA. Most had acute or chronic leukaemia. IC: A total of 25 pts had proven candidemia; two pts each had chronic disseminated candidiasis (CDC, one proven/one probable) or other disseminated *Candida* infections (one proven/one probable). Favourable response was noted in 62% (18/29), including a 58% (15/26) response as first-line Rx and a 100% (three of three) response as salvage Rx. Success in candidaemia was 68% (17/25); success in CDC and other disseminated infections was 25% (one of four). Outcome across the different *Candida* species was similar. IA: 35 (76%) pts had pulmonary IA (15 proven, 20 probable). Other sites of infection (all proven) included sinus (15%), skin (2%), and disseminated (7%). Favourable responses were noted in 35% (16/46), including a 42% (five of 12) response as first-line Rx and 32% (11/34) response as salvage Rx. Success by site of infection was pulmonary 34% (12/35), sinus 43% (3/7), and skin/disseminated 25% (1/4).
Conclusions: Review of the CAS database demonstrates that CAS is effective in neutropenic pts with either proven/probable cases of IC or IA.

O423 Impact of resolution of fever on the overall composite endpoint in a phase III study of caspofungin vs. liposomal amphotericin B as empirical therapy for neutropenic patients with persistent fever

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Objectives: An exploratory analysis was conducted within a recent phase III trial of caspofungin (CAS) vs. liposomal amphotericin B (L-AmB) to determine the impact of the endpoint definition for the resolution of fever (RF) on the overall response rate using a five-part composite endpoint.
Methods: The primary analysis used a five-part composite endpoint: successful outcome of baseline invasive fungal infection (IFI), no breakthrough IFI to 7 day post-Rx, survival to 7 day post-Rx, no discontinuation of study drug for toxicity or lack of efficacy, and RF defined as afebrile (<38°C) for 48 h during neutropenia and before the end of study Rx. A favourable response required success in each endpoint component. This analysis demonstrated noninferiority of CAS to L-AmB. Subsequently, three prespecified exploratory analyses were performed using less conservative definitions for RF: 24 h afebrile during neutropenia before end of Rx, afebrile at 7 day post-Rx, and exclusion of RF from the composite analysis. All other endpoint components were handled consistently with the primary analysis. The impact of risk category, a stratification parameter at study entry, was examined. High-risk patients (pts) had allogeneic haematopoietic transplant or relapsed acute leukaemia; all others were low-risk.
Results: Stratum-adjusted overall response rates are shown in the Table 1 below. In each analysis, CAS met noninferiority criteria. When RF was excluded from the composite endpoint, CAS was superior to L-AmB. Low-risk pts in both Rx groups had shorter duration of neutropenia (by 3–4 days). Due to less opportunity to demonstrate 48 h afebrile during a shorter period of neutropenia, low risk patients failed the RF endpoint in the primary analysis more often than high-risk pts (RF rates 37–39% for low vs. 50–52% for high risk). In the exploratory analyses, a greater increase in response rates was seen for low than high-risk pts.

Table 1. Overall Response Rate (%) by Definition of RF

	48 hr afebrile while neutropenic	24 hr afebrile while neutropenic	7-day post-Rx	Exclude RF
CAS	33.9	51.6	55.3	81.7
L-AmB	33.7	47.8	53.5	74.7
Difference	0.2	3.8	1.8	7.0
(95.2% CI)	(−5.6, 6.0)	(−2.4, 9.9)	(−4.3, 8.0)	(1.9, 12.1)

Conclusions: Overall response rates for the composite endpoint in this empirical therapy study are driven by low response rates for RF, especially in low-risk pts due to their shorter duration of neutropenia. Noninferiority of CAS to L-AmB was confirmed in each analysis. When RF was not included as part of the composite endpoint, CAS was superior to L-AmB in the empirical therapy of persistently febrile neutropenic pts.

O424 Monitoring of Epstein-Barr virus (EBV) DNA load after haematopoietic stem-cell transplantation for prevention, early diagnosis as well as antiviral and immune therapy of EBV-associated lymphoproliferative diseases

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Objective: The development of a life-threatening EBV-associated lymphoproliferative disease (LPD) is a serious complication in patients after haematopoietic stem-cell transplantation (HSCT). Monitoring of viral load is a useful and sensitive parameter in the surveillance of EBV reactivation for prevention and treatment of EBV-associated LPD.

Methods: A semiquantitative PCR for evaluating EBV-genome copy numbers in plasma and peripheral blood mononuclear cells (PBMC) was established. The method bases on a nested PCR using primers of the structural protein region p23 and an end-point dilution. Using this assay in 65 patients undergoing HSCT EBV DNA load was prospectively screened weekly after transplantation.
Results: EBV reactivations (>1000 EBV-genome copies measured in 100 000 PBMC) were observed in 11 patients (16.9%). Three patients developed LPD with extremely high EBV-genome copy numbers in PBMC (>100 000) and plasma. The rapid increase of EBV-genome copies occurred 1–4 weeks before the onset of the disease. After combined antiviral and immune therapy two of three patients showed a dramatic decrease of EBV load and survived, while the third patient died of lymphoma. A subclinical EBV reactivation was observed in five cases with EBV-genome copies ranging from 1000–10 000. After reduction of immunosuppression the EBV levels normalised. In three patients the high copy number of >10 000 and plasma positivity prompted the physicians to start pre-emptive therapy with rituximab and cidofovir for prevention of EBV-associated LPD. After drug administration the high EBV load in plasma and PBMC reduced dramatically. The decrease of EBV-genome copies was associated with a decrease of B lymphocytes and an increase of CD8+ and CD4+ T lymphocytes. The 54 patients who had copy numbers of <1000 did not develop EBV-associated LPD.
Conclusions: Monitoring of EBV DNA load is a useful method for early diagnosis and treatment of EBV-associated LPD and for follow-up the efficacy of therapy.

O425 Real-time PCR quantification of EBV, CMV and HHV6 in patients after allogeneic haematopoietic stem cell transplantation

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Objectives: Herpesvirus infections cause serious complications in patients after haematopoietic stem-cell transplantation (HSCT). Recently, real-time PCR monitoring of viral load has become the method of choice for distinguishing between benign and life-threatening herpesvirus infections and thus for indication of antiviral therapy. The aim was to investigate the frequency and outcome of infection with EBV, CMV and HHV6 in a consecutive group of children undergoing allogeneic HSCT.
Methods: We tested 1566 samples collected from 58 children transplanted between January 2001 and August 2003. The samples were drawn weekly by day +100 after HSCT, and biweekly thereafter until day +365. DNA was extracted from whole blood, and tested for EBV, CMV and HHV6 by real-time PCR techniques. The viral load was normalised to 100 000 human genomic equivalents (GE) as assessed by albumin gene quantification. A provisional cut-off level of the quantity was set to 1000 copies of viral DNA per 100 000 GE.
Results: The quantity threshold for either of the three viruses was crossed in 17 of 58 (29%) children. The proportion of children with EBV, CMV and HHV6 activation was identical, Eight of 58 (14%) for each virus. Three children required anti-CD20 administration due to prolonged or rising EBV viral load. No child died of EBV-related complications. Therapy with foscavir or ganciclovir was instituted in seven of eight CMV-positive patients. Five of the patients experienced repeatedly treated reactivations, one of them died of CMV pneumonia. One child had CNS symptoms attributable to HHV6 infection (documented in blood and cerebrospinal fluid), and was therefore treated with acyclovir and ganciclovir. All remaining children with HHV6 had long lasting low-to-intermediate viral loads without detectable effects on the post-transplant course.
Conclusion: Our report confirms usefulness of frequent monitoring of EBV and CMV viral load in routine post-transplant care. Since we had instituted the quantitative monitoring, no patient died of post-transplant EBV lymphoproliferation (EBV-LPD) in contrast to four deaths of EBV-LPD in the preceding year. To our best knowledge, this is the first report on longitudinal monitoring of HHV6 in an unselected consecutive group of children after allogeneic HSCT, showing that HHV6 frequently accompanies, but rarely complicates the post-transplant course. Supported by the Ministry of Health grant 7459.

O426 Contamination of bone marrow products with a *Mycobacterium mucogenicum*-related pathogen

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Background: Contamination of bone marrow products (BMP) occurs in 8–20% of cases, mostly by nonpathogenic microorganisms, usually without significant morbidity among bone marrow transplant (BMT) recipients. Contamination can occur in the course of BMP collection or during processing for cryopreservation. BMP contamination with mycobacteria is rare and poses a significant diagnostic and therapeutic challenge. In our institution, samples of BMP are cultured before cryopreservation as a part of quality assurance process. Usually these samples are incubated for 5–6 days.
Objectives: To describe an outbreak of contamination of BMP by an unusual microorganism, the microbiological identification and infection control measures taken to investigate and contain the outbreak.
Methods: BMP samples were inoculated into aerobic bottles and cultured using a continuously monitored broth system (BACTEC). Initial identification was carried out by subculturing on 5% sheep blood agar Gram and Ziehl-Nielsen staining. Final identification was carried out by PCR-restriction fragment length polymorphism (RFLP) analysis of the 65-kDa heat shock protein gene and sequencing of 16s rRNA gene. BMP preservation procedure practices were observed, and environmental samples were cultured.
Results: The first case was incidentally detected after prolonged incubation. Following that, subsequent samples were incubated for at least 10 days. A Gram-positive rod was isolated from five of 45 BMP samples during May–July, 2003. Sequencing of the pathogen 16s rRNA revealed 90% identity to the published sequence of *Mycobacterium mucogenicum*. During the BMP processing, ice cubes generated by ice machine using general water supply were used for cooling. The same mycobacterium was isolated from the ice machine, ice cubes and tap water. Substitution of ice cubes by cooling trays, aborted the outbreak. Two patients were transplanted by the contaminated BMP with no clinical and microbiological consequences.
Conclusion: Water may be a source of mycobacterial contamination of BMP. BMP samples should be routinely cultured before cryopreservation and incubated for a prolonged period. Molecular methods are invaluable tools for identifying unusual pathogens. A thorough epidemiological investigation is essential for controlling an outbreak.

O427 Lack of association between CMV-viraemia and bronchiolitis obliterans syndrome in lung transplant recipients

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Purpose: CMV infection is considered by many to be one of the probable risk factors for developing BOS. Published evidence is

controversial. Some transplant centres employ aggressive CMV-prophylaxis regimes to prevent early or late CMV-related complications. We describe our experience with prospective CMV surveillance using quantitative PCR (QPCR) and the subsequent development of BOS.
Method: Case note survey of adult lung transplant recipients (LTx) between January 2000 and November 2001. Patients who were CMV-positive prior to transplantation (IgG+) and CMV mismatches (MM = D+/R-) were followed with weekly CMV QPCR. MM received prophylaxis with oral ganciclovir for 3 months. Spirometry and flow volume measurements were performed at regular intervals. Development of BOS was assessed by ISHLT criteria during follow-up period (22–45 months). Bronchoalveolar lavage and transbronchial biopsies were performed at 1, 4, 12, 26 and 52 weeks and whenever clinically indicated. CMV D-/R- LTx served as control group. Recipients were divided into three groups according to CMV QPCR values (copies/ml) (i) 10^3–10^4, (ii) 10^4–10^5 and (iii) 10^5–10^7.
Results: Nine of 50 patients were MM, eight of these had positive QPCRs: one in group (i), three in (ii), and four in (iii). Three patients developed CMV disease and were treated. One asymptomatic patient in group (iii) developed BOS at 6 months. 16 of 22 IgG+ patients were QPCR-positive: Five in group (i), six in (ii), and five in (iii). Five patients were symptomatic, four of these were treated. Three asymptomatic patients (one QPCR-negative, two in group (i) developed BOS at 20, 29 and 6 months, respectively. Five of 19 patients from the D-/R- control group developed BOS.
Conclusion: We did not find any correlation between either CMV-QPCR results or CMV disease and BOS development in our surveillance group. Although the figures are small, we are encouraged that oral ganciclovir prophylaxis is only justified to protect the CMV-MM high risk group.

O428 Correlation between HHV8 infection and Kaposi's sarcoma in a group of heart and lung transplant recipients

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Objective: The aim of this retrospective study was to evaluate the seroprevalence of human herpesvirus 8 (HHV8) in a group of 77 solid organ recipients (63 heart, 13 lung and one heart–lung) transplanted at our Division of Cardiosurgery from 1997 to 2002 and in 62 donors, to detect seroconversion after transplantation and to assess the risk of developing KS in pretransplant positive patients, post-transplant seroconverted patients and seronegative patients.
Methods: Serum samples of recipients and donors, collected before transplantation, were tested by an immunofluorescence assay based on BCBL-1 cell line to detect HHV-8 anti-lytic antibodies. After transplantation at least one serum sample was tested for each patient seronegative before transplant to detect seroconversion. Diagnosis of KS was made clinically during follow-up visits and confirmed by hystological examination.
Results: Of the 77 pretransplant serum samples, 13 (16.9 %) were positive for HHV8 antibodies. The donor's serum was tested in 62 cases and resulted positive in five (8%). Post-transplant seroconversion was observed in 15 organ recipients. Among these four received an organ from a seropositive donor (D+/R-). Only one of the mismatched patients is still seronegative (follow-up of 8 months). Four of 13 patients who were HHV8-seropositive before transplantation developed KS, corresponding to an incidence of 30.7%; in these patients the immunosuppressive regimen was standard with cyclosporin or tacrolimus plus prednisone and the median interval between transplantation and diagnosis of KS was 2 months (range 1–35 months). However none of the 49 seronegative patients and none of the 15 seroconverted patients at the moment have developed clinical KS (medium follow-up of 35.7 months).
Conclusion: This study suggests that HHV8 antibody detection is a useful means to recognise heart and lung transplanted patients at higher risk of KS, particularly in geographical areas where seroprevalence is higher than average, because HHV8 seropositivity appears to have predictive value for an early onset of KS. However, D+/R- mismatch does not seem to represent a risk of iatrogenic KS. Reducing the degree of immunosuppression prematurely could play a critical role in preventing KS in HHV8-infected recipients.

O429 Documented bacterial and fungal infections in orthotopic liver transplant recipients: an Italian multicentre study in 416 patients

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Objectives: The aim of this study was to understand the natural history of infection in liver transplant recipients.
Methods: We prospectively studied incidence, main clinical characteristics, aetiology and outcome of documented infections developing during the first 4 months after transplant in 416 patients. who underwent liver transplantation from 1999 to 2001 in 10 liver transplant centers in Italy.
Results: The patients' mean age was 46 years and the UNOS score was 1 in 5% (22/416), 2a in 46% (192/416), 2b in 1.7% (109/416), and 3 in 21% (86/416) of the patients (21% missing data). Among patients with liver cirrhosis, 12% were Child A, 25%, Child B and 44% Child C. Sixty-one of 416 patients (15%) received a split transplant, and 328 (78%) a whole transplant (27 missing). The overall number of patients who had at least one documented infection was 162/416 (39%). A total of 287 episodes were identified, according to strict clinical and microbiological definitions (6% of the patients had four or more episodes). Of 287 isolated pathogens, 147 (51%) were Gram-positive cocci, 115 (40%) Gram-negative rods and 25 (9%) fungi. Enterococci, staphylococci and *Pseudomonas* accounted for 21, 28 and 23% of all pathogens isolated. The overall crude mortality rate at 120 days after transplantation was 12% (50/416).
Conclusions: The infection was considered to be the main or an associated cause of death in 28/416 patients (7%), while the remaining 22 patients died from other reasons.